AN ASSESSMENT OF THE MARICULTURE POTENTIAL OF INDIGENOUS EASTERN CARIBBEAN BRINE SHRIMP

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An Assessment of the Mariculture Potential of Indigenous Eastern Caribbean Brine Shrimp

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CONTENTS

LIST OF TABLES

TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

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EXECUTIVE SUMMARY

A study has been undertaken to provide a technical basis for evaluating development potential for culturing endemic brine shrimp in Eastern Caribbean salt ponds. Salt ponds in Anguilla, Antigua, the British Virgin Islands, and St. Kitts have been characterized in terms of *Artemia* distribution and physical factors. 25% of these ponds have endemic brine shrimp populations. Significant quantities of polyunsaturated long-chain fatty acids (PUFA) were found in some *Artemia*, indicating the suitability of these animals as food for mariculture operations. Fatty acid profiles of algae cultured from hypersaline ponds indicate that these algae are promising as food sources for improving the concentration of PUFA in *Artemia*.

Electrophoretic studies suggest that Eastern Caribbean *Artemia* probably represent a distinctive strain. *Artemia* populations in St. Kitts show an increase in the proportion of cyst bearing females two to four days after sudden drops in salinity. *In vitro* experiments similarly indicated a significant increase in cyst production after salinity reduction. At high salinities, live-bearing females were more abundant than cyst-bearers. These findings are in contrast to studies conducted on *Artemia* from other parts of the world.

Laboratory and pond studies indicate that optimum salinity and temperature conditions for St. Kitts *Artemia* lie in the range 150 - 180 o/oo and 25 - 32 C. A negative correlation was observed between salinity and population size above 200 o/oo. With a single exception, maximum population size in experimental ponds was obtained at salinities ranging from 160 to 185 o/oo. Under these conditions, the estimated density of brine shrimp was 3900 per square meter. Natural pond production in St. Kitts compares favorably with that of other locales, but requires enhancement to equal the production of intensive culture facilities.

While attempts to optimize production of *Artemia* cysts in experimental ponds were not successful, there is no indication that future manipulations will be similarly ineffective. Fertilization of ponds has considerable potential, and algae with favorable concentrations of PUFA have been shown to respond to increased concentrations of ammonia nitrogen. Brewery grain waste is particularly rich in this nutrient.

Evaluation of the development potential for Eastern Caribbean Artemia indicates that brine shrimp culture in endemic salt ponds can be a viable proposition, particularly as a cottage industry. It is suggested that one or more pilot projects be established with the intention of developing a commercially viable operation. An appropriate model for such an operation is described with suggestions for an optimization program. It is particularly urgent that the Eastern Caribbean Artemia gene pool be conserved, in view of the precarious future of natural brine shrimp habitats.

I. INTRODUCTION

In recent years, there has been growing interest in the development of marine food resources among small islands in the Eastern Caribbean. These resources, however, are limited, and while there is substantial local interest in fisheries development, there is also concern that local stocks are in danger of overexploitation (Goodwin and Taylor, 1980). As a result, increasing attention is being focussed on aquaculture to supplement harvest of natural stocks. But despite widely publicized success stories, many aquaculture efforts are hampered by the lack of a reliable food supply for various species being cultured. One of the most satisfactory and widely used foods in aquaculture is the brine shrimp *Artemia*.

Brine shrimp are branchiopod crustaceans whose natural habitat is hypersaline ponds, salinas, and lagoons. *Artemia* are able to tolerate osmotic conditions (salinity in excess of 250 o/oo) which preclude all other animal life except the brine fly *Ephydra*. Brine shrimp are obligate filter feeders, and ingest particulate matter ranging in size from 5 to 50 microns (Dobbelier, *et al*., 1980). Reproduction may be via live births from internally hatched eggs (ovoviviparous) or via cysts (oviparous). Reproductive mode depends upon environmental conditions; salinity and dissolved oxygen are most often cited as determining factors. In the ovoviviparous mode, great biomass production is possible in natural salt ponds with reports of 1.5 metric tons/ha/mo. Reliable supplies of live or frozen brine shrimp can contribute significantly to the development of aquaculture in the Eastern Caribbean. It is cyst production, however, which has received the greatest international attention for harvest and exploitation.

Female *Artemia* may produce up to 300 cysts per brood and may hatch broods as often as every five days. These cysts float to the pond surface where they are blown to the water's edge, often in enormous quantities. Cysts are resistant to extreme conditions, and remain viable over a temperature range of -273 to 100 C (Skoultchi and Morowitz, 1964, and Hinton, 1954), after prolonged storage under vacuum, or after ten years' immersion in alcohol (Iwanami, 1984), and retain hatch competence in the digestive tracts of birds for at least three days (MacDonald, 1980). They are relatively easy to hatch, and recent decapsulation techniques (removal of the cyst's tough exterior) have provided for greater hatching percentages (Bruggeman *et al*., 1980).

The ease with which Artemia cysts can be stored, collected, and hatched has made them a preferred experimental animal for a variety of laboratory investigations (Sorgeloos, 1980) and as food for more than 85% of the marine organisms cultivated to date (Kinne, 1976). As a result, there is an extensive literature on various aspects of brine shrimp biology (Littlepage and McGinley, 1965; Sorgeloos et al. 1980). Ecological studies, however, have been extremely scanty with respect to Caribbean Artemia habitats. This constitutes a major obstacle to further progress in the practical use of brine shrimp for aquaculture in the region. The importance of such data is apparent when one considers the physiological diversity to be found within the genus Artemia. For example, reproduction is dioecious (two sexes) in most cases, but strains of parthenogenic brine shrimp have been reported in India (Kulkarni, 1953). Similarly, brine shrimp in northern temperate sites produce living young at low salinities and cysts at higher salinities, while brine shrimp in the Netherlands Antilles reproduce in an opposite fashion (Kristensen and Hulscher-Eweis, 1972). Moreover, while the genus Artemia was once thought to contain only the species salina, there are now believed to be at least five species (Bowen, et al., 1980), some of which are quite dissimilar in their ecological requirements and suitability for aquaculture.

These differences are particularly significant when one considers the precarious future of Eastern Caribbean Artemia habitats; i.e., endemic salt ponds. Over 200 of these ponds have been identified in the northeastern Caribbean, most of which are generally considered to be of little or no value. A very few are used for salt production. The rest are considered to be most appropriate for sand extraction, conversion into marinas, solid waste dumps, or landfill areas. The latter views are becoming more prevalent with the recent boom in tourism and consequent increased pressure for filling salt ponds. In addition to destroying the natural function of salt ponds as sediment traps which protect coral reefs and coastal areas from direct terrestrial runoff, elimination of salt ponds also means elimination of indigenous (and possibly unique) strains of Artemia. The importance of preserving these strains has been summarized by Persoone and Sorgeloos (1980):

> We should be aware that if we destroy the original Artemia gene pools we condemn at the same time our basic potential of genetic improvement and cross-breeding of Artemia strains. And this, exactly as in any type of farming and husbandry, would be the biggest drawback for all the hopes which we are

placing today in the advancement of aquaculture and the mass culturing of *Artemia* as a most welcome addition to the production of animal protein.

The importance of exploring the opportunities to develop new sources of *Artemia* in the Eastern Caribbean is underscored by the fact that brine shrimp from several major producers have recently been found to be of questionable quality. In a biological testing program of various geographical strains conducted by the U. S. Environmental Protection Agency, it was found that certain samples produced excellent survival and growth, while others resulted in mortalities up to 100% (Johns *et al*., 1980; Klein-Macphee *et al*., 1980). Brine shrimp shortages and quality are a serious hindrance to aquaculture programs generally, and it has been estimated that in 1984 total production of brine shrimp will only be sufficient to meet half the world demand.

Brine shrimp in the Eastern Caribbean represent an underutilized resource with considerable potential. Investigation of this potential is particularly timely in light of:

* the world demand for brine shrimp
* the growing interest in mariculture
* the importance of these animals to many proven culture systems
* the lack of biochemical data for local strains
* the lack of biophysical data on Eastern Caribbean salt ponds
* the need to maximize the use of indigenous resources in small islands

The objective of this project was to provide a technical basis for evaluating development potential for culturing endemic brine shrimp in Eastern Caribbean salt ponds by:

1. determining the nutritional value of *Artemia* reared on phytoplankton cultures derived from Eastern Caribbean salt ponds;

2. determining fecundity and reproductive mode of brine shrimp produced at selected levels of temperature, salinity, and dissolved oxygen; and

3. investigating possible correlations between selected biotic/abiotic characteristics of *Artemia* habitats and reproductive activity of *Artemia* populations in certain Eastern Caribbean salt ponds.

The present study was directed toward four hypotheses:

1. That *Artemia* is endemic to selected Eastern Caribbean salt ponds and when reared on phytoplankton cultures derived from the same ponds have nutritional value which is at least equal to reference *Artemia* reared on standard diets.

2. That the reproductive mode (ovoviviparous or oviparous) of Eastern Caribbean *Artemia* will vary when these animals are reared at different levels of temperature, salinity, and dissolved oxygen.

3. That the size of *Artemia* populations and reproductive mode in Eastern Caribbean species vary with changes in one or more of these factors: temperature, salinity, dissolved oxygen, nitrates, nitrites, ammonia, or phosphate.

4. That natural salt ponds may be modified to produce levels of factors tested in hypothesis "3" which optimize production of *Artemia* cysts.

Four major areas of activity were undertaken to test these hypotheses:

1. Characterization of endemic salt ponds in order (a) to assess variability among salt pond sites within the study region; and (b) to identify correlations between selected physical/chemical factors and *Artemia* biomass and reproductive mode

2. Laboratory investigation of the response of Eastern Caribbean *Artemia* to variations in temperature, salinity and dissolved oxygen

3. Biochemical analyses (a) to assess relative nutritional value of *Artemia* reared on natural diets; (b) to provide additional biochemical characterization of native *Artemia* relative to other strains; and (c) to spot check selected samples for organophosphate and chlorinated hydrocarbon contamination

4. Investigation of options for manipulating salt ponds to optimize the production of *Artemia* biomass and cysts having acceptable nutritional characteristics

II MATERIALS AND METHODS

A. Investigation of Selected Eastern Caribbean Salt Ponds

1. Preliminary Survey

An initial survey of natural salt ponds in the Eastern Caribbean was undertaken to locate *Artemia* populations, determine the suitability of various sites for further investigation, and to provide baseline information for subsequent data collection. In order to provide a broader understanding of seasonal or ephemeral changes in salt ponds as *Artemia* habitats, ponds were visited both during the rainy season (November-December, 1983) and the dry season (May-August, 1984). The following sites were visited during this study:

Anguilla (Figure 2) -- Road Pond, West End Pond, Maunday's Pond, Meads Bay Pond, Cove Bay Pond, Mt. Fortune Pond, Savannah Bay Pond, Long Salt Pond, Rendezvous Pond

Antigua (Figure 3) -- McKinnon's Pond, Cocks Hill Pond, Jolly Hill Pond, Deep Bay Pond, York's Pond, Pinching Bay Pond, Galley Bay Pond

British Virgin Islands:

Virgin Gorda (Figure 4) -- Pond Bay

Prickly Pear (Figure 5) -- Brandy Pt., Biras Creek

Tortola (Figure 5) -- Pockwood Pond, Belmont Pond, Cane Garden Pond, Josia's Bay

Beef Island (Figure 5) -- Goose Hole Pond, Bluff Bay Pond, Trellis Bay Pond, Sprat Point Pond, Conch Bay Pond

Salt Island (Figure 5) -- Large Pond, Small Pond

Anegada (Figure 6) -- East End Pond, Flamingo Pond, Bones Bight Pond, Red Pond

St Kitts (Figure 7) -- Half Moon Pond, Frigate Bay Pond, Little Salt Pond, Great Salt Pond, Unnamed Pond

During initial surveys, accessible shorelines of each pond were examined for the presence of *Artemia* and cysts. Where the pond bottom was firm enough to support weight, a nylon dip net (202 u mesh) was dragged through the pond and subsequently examined for entrapped *Artemia* or

Figure 1 -- The Eastern Caribbean

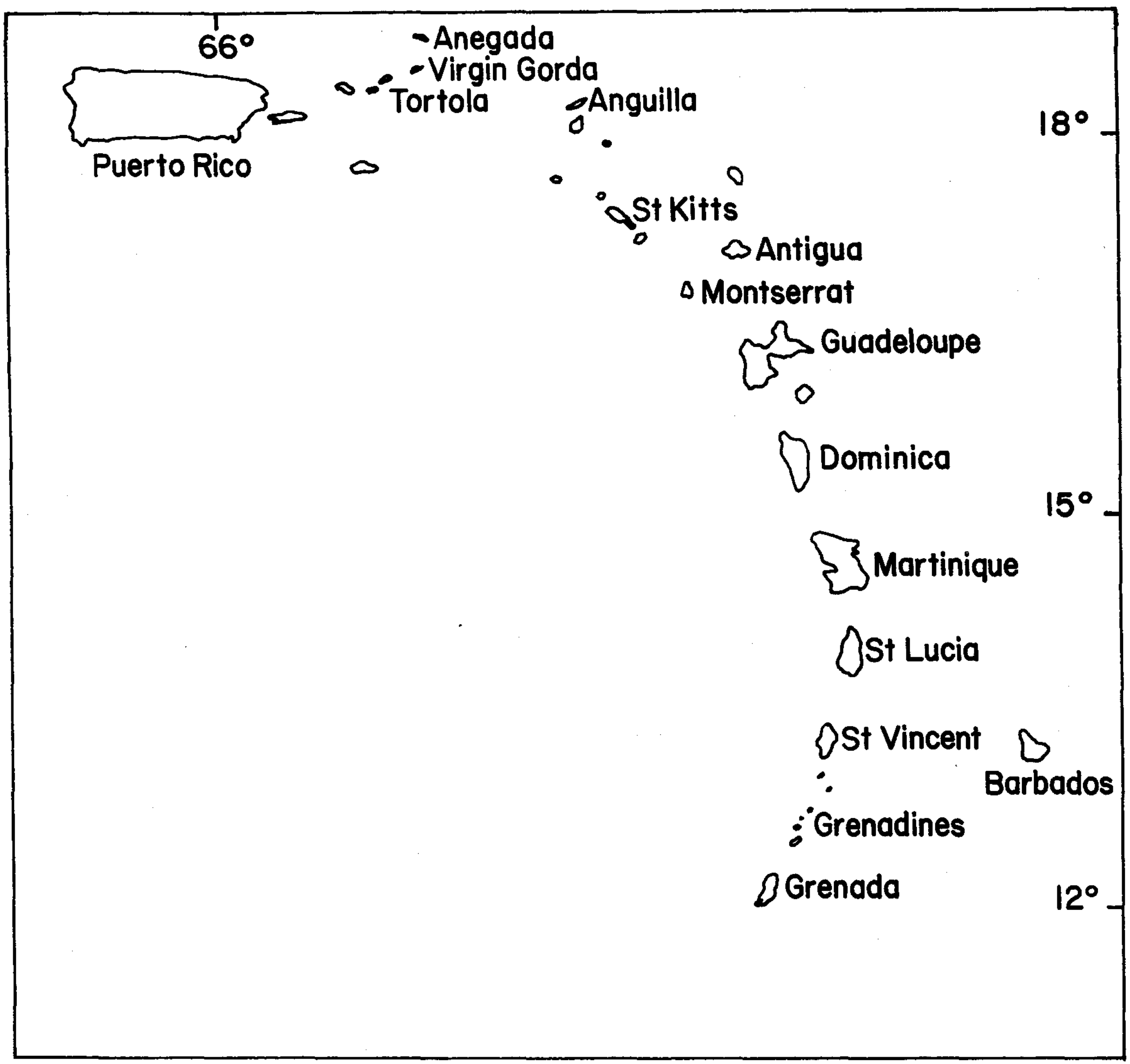


Figure 2 --- Sampling Sites in Anguilla (adapted from Eastern Caribbean Natural Area Management Program, Preliminary Data Atlas, 1980)

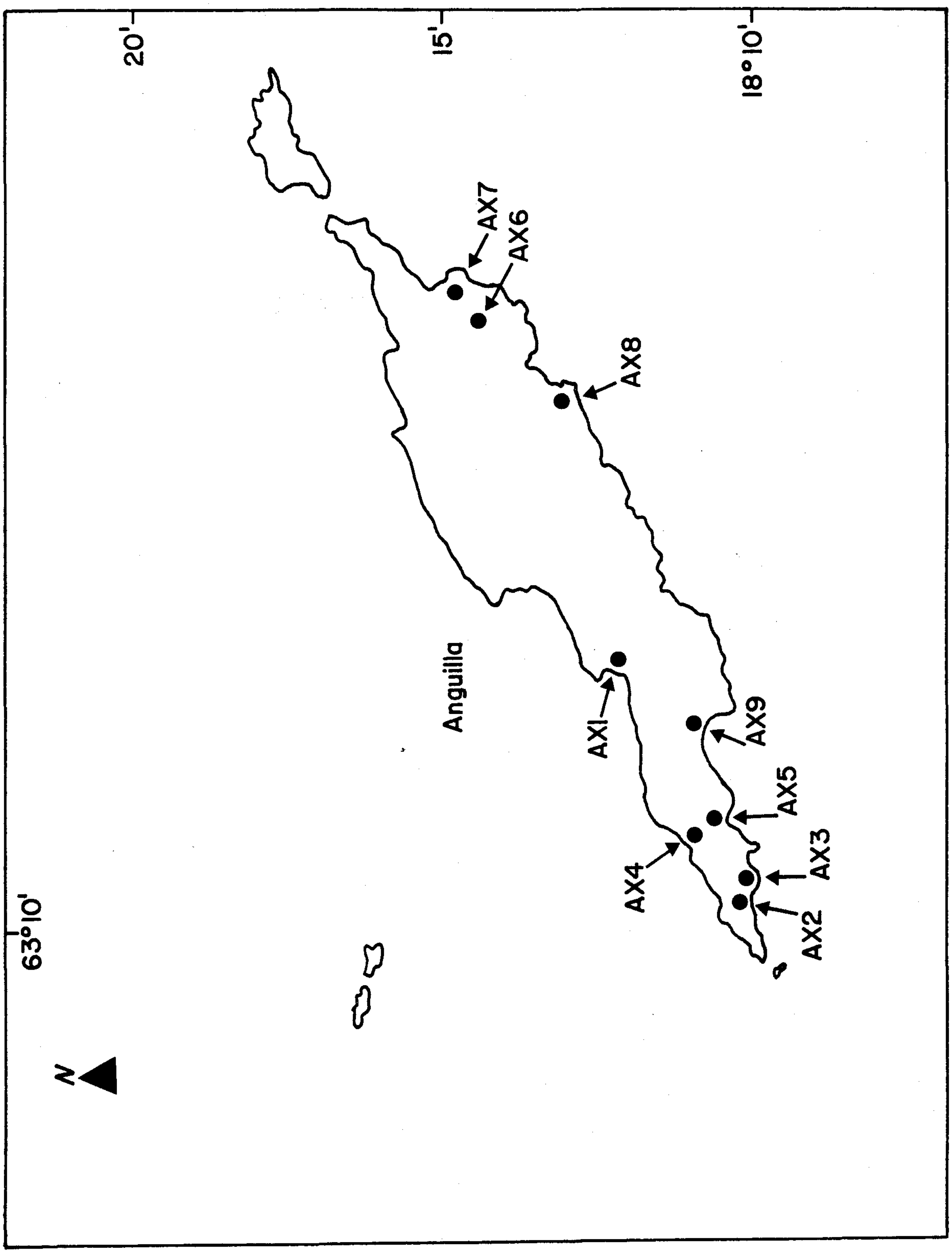


Figure 3 -- Sampling Sites in Antigua (adapted from Eastern Caribbean Natural Area Management Program, Preliminary Data Atlas, 1980)

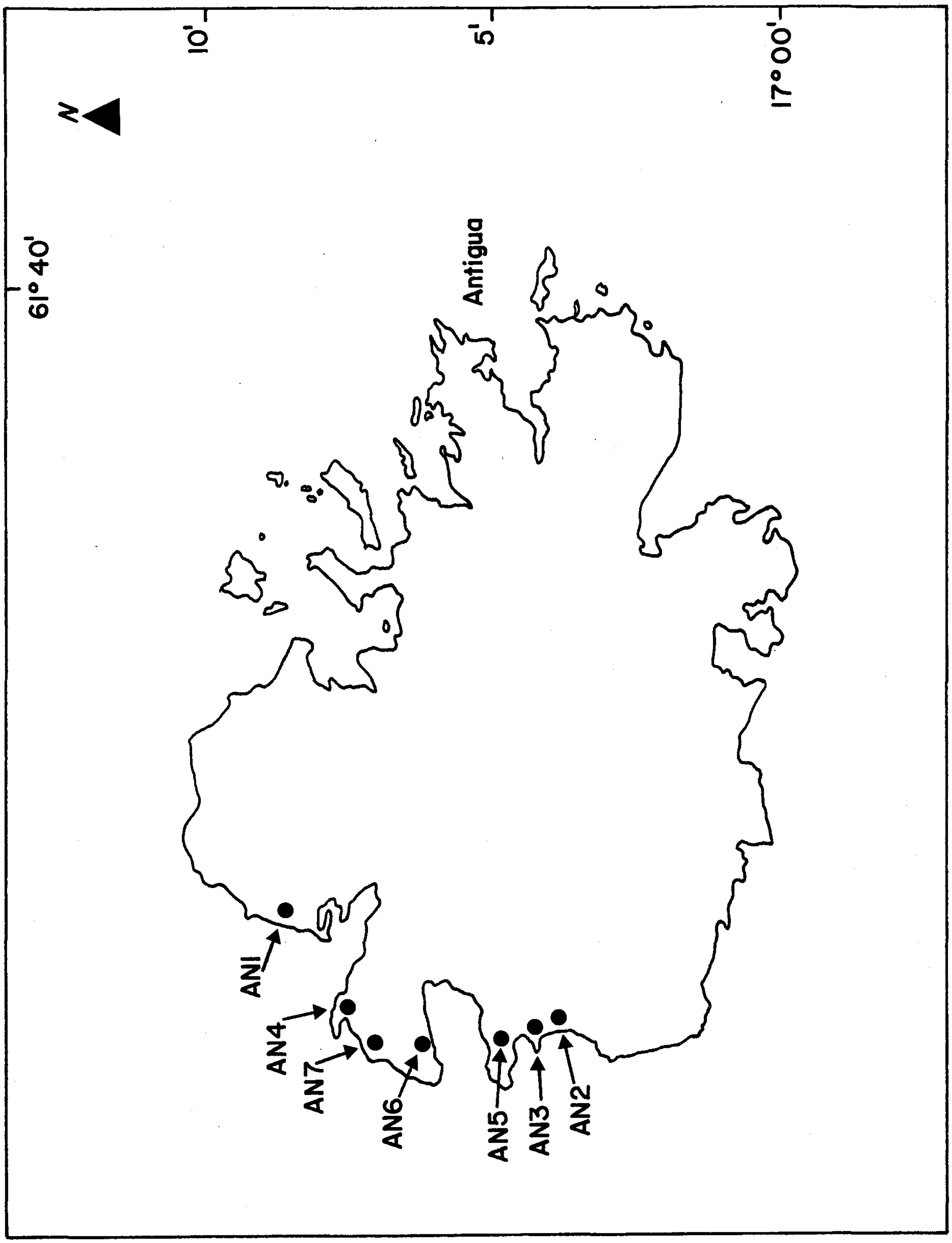


Figure 4 -- Sampling Sites in Virgin Gorda (adapted from Eastern Caribbean Natural Area Management Program, Preliminary Data Atlas, 1980)

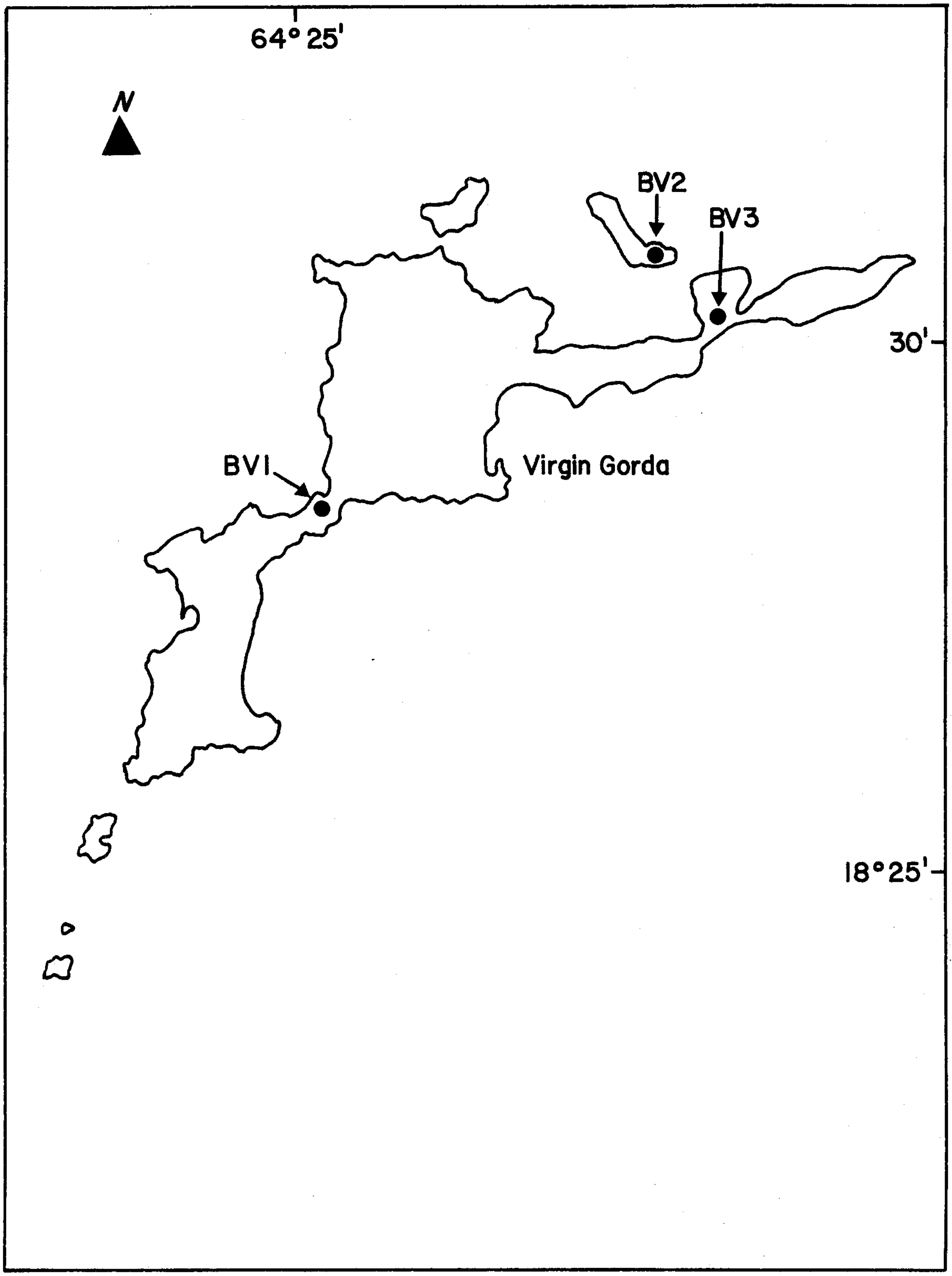


Figure 5 -- Sampling Sites in Prickley Pear, Tortola,, Salt Island, and Beef Island (adapted from Eastern Caribbean Natural Area Management Program, Preliminary Data Atlas, 1980)

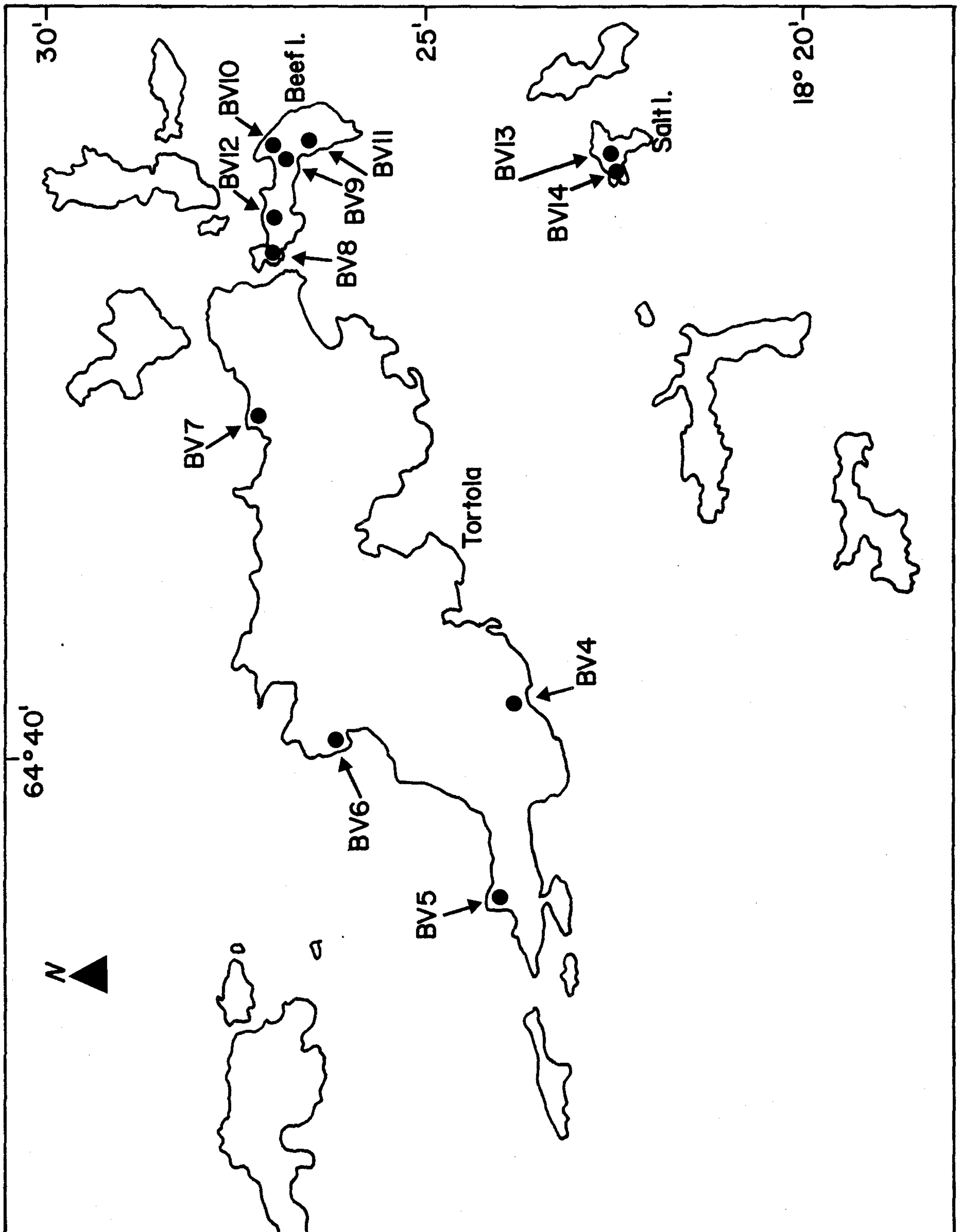


Figure 6 -- Sampling Sites in Anegada (adapted from Eastern Caribbean Natural Area Management Program, Preliminary Data Atlas, 1980)

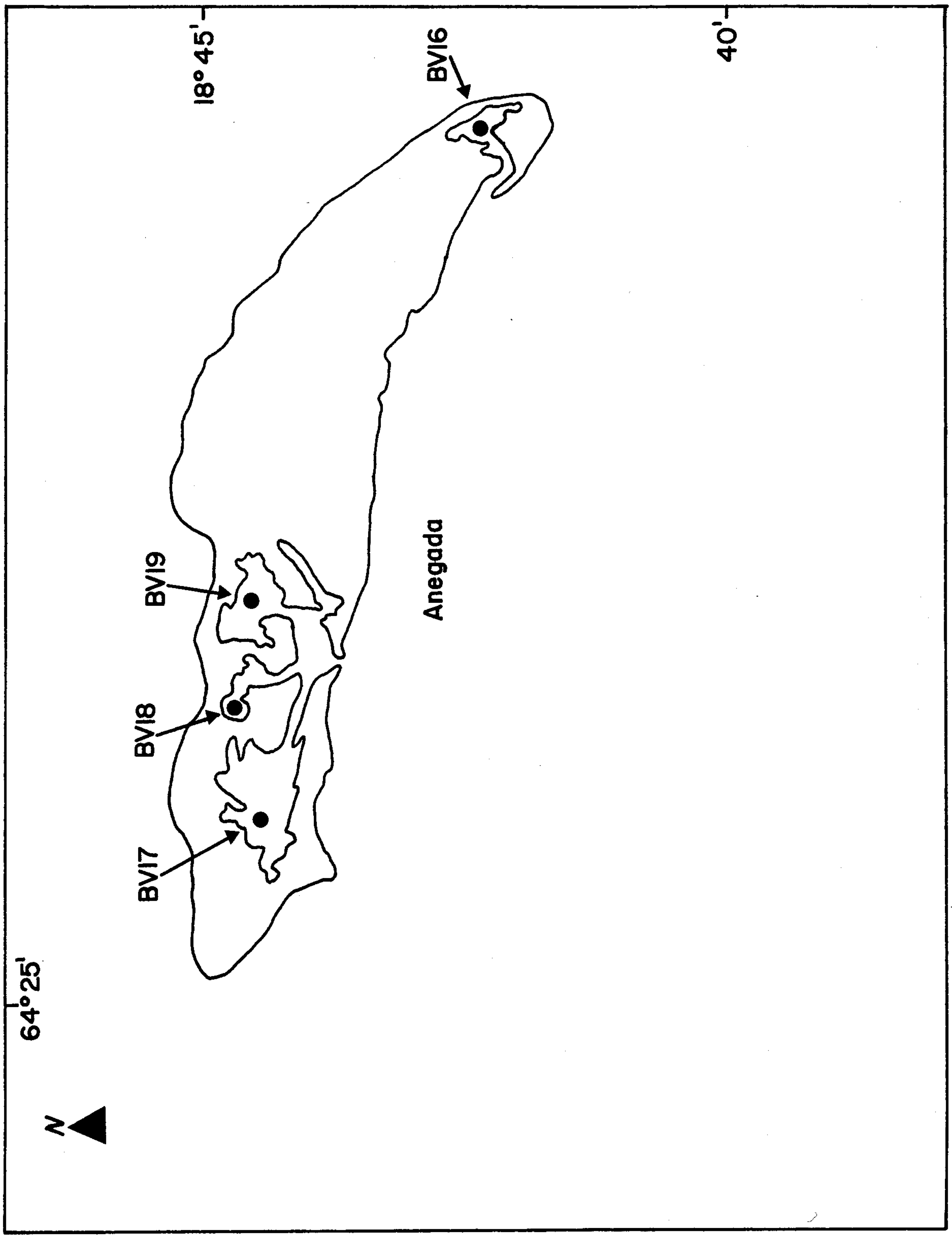
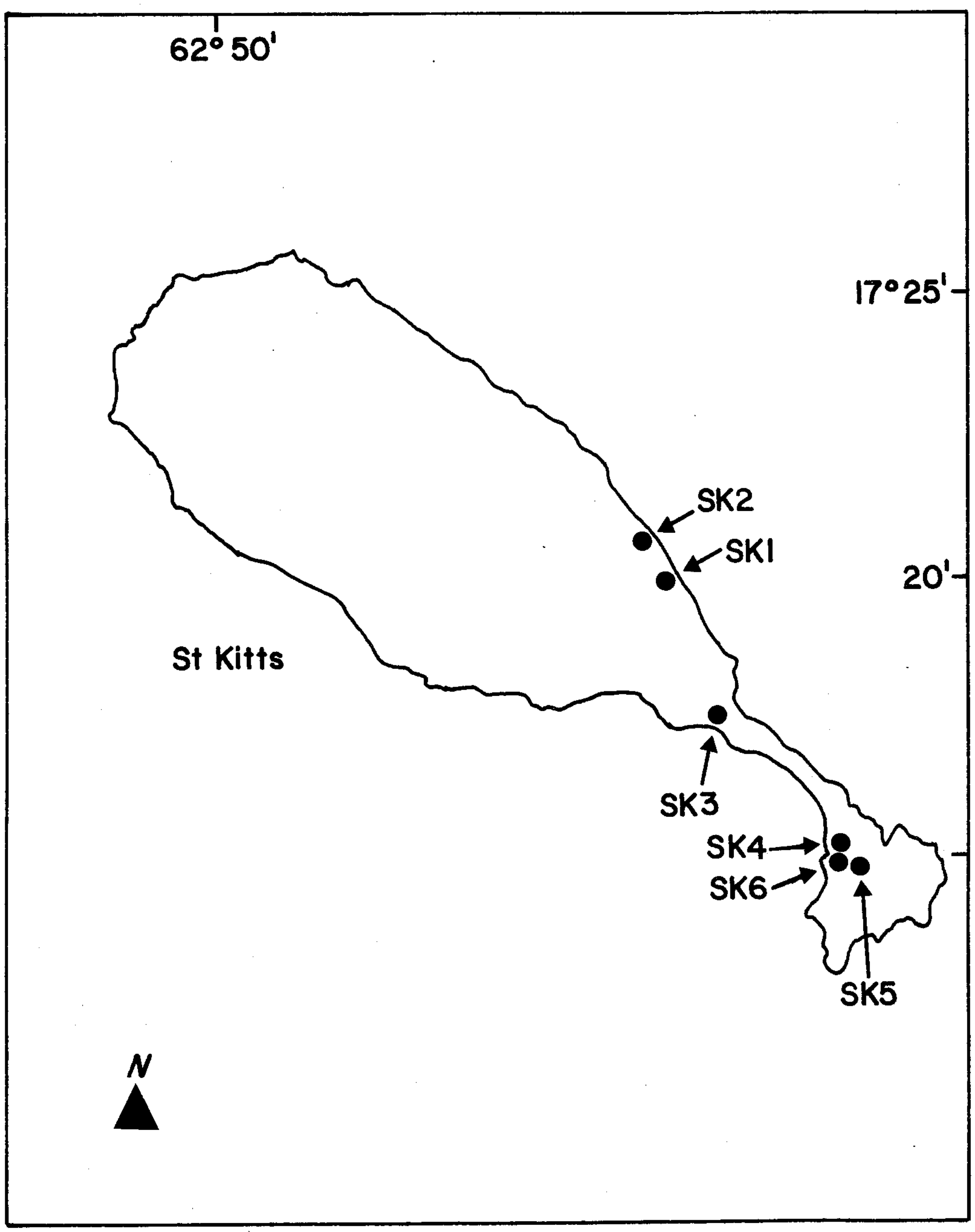


Figure 7 -- Sampling Sites in St. Kitts (adapted from Eastern Caribbean Natural Area Management Program, Preliminary Data Atlas, 1980)



cysts. If live Artemia or cysts were not found by these procedures, the pond was considered devoid of brine shrimp.

Artemia collected by the above procedure were transferred to a solution of 10% formalin in pond water and retained for fatty acid analyses described below.

Cysts collected during the surveys were transferred to polypropylene jars containing pond water saturated with table salt. Cysts were subsequently processed for storage using the method described by Vos and de la Rosa (1980). Cysts in saturated brine were aerated for 24 hours, rinsed over a 160 u sieve with tapwater, and transferred to a separatory funnel containing tapwater. Cysts were then aerated for 10 minutes, after which viable cysts were allowed to settle (empty cysts float during this procedure). Viable cysts were drawn off, blotted, and air dried at 30 - 35 c for 24 hr. Dried cysts were stored in polypropylene jars for analysis or later use. As an alternative storage procedure, some cysts were returned to saturated brine after the above procedure was completed.

Water salinity in each pond was determined with a refractometer (American Optical Company). Where pond salinity exceeded the range of the instrument, a 1:1 dilution of the sample was prepared with distilled water. pH was determined with a Hach model 16400 pH meter. Temperature was determined to the nearest degree with a mercury thermometer. When possible, water samples for these measurements were taken at least 15 cm below the water surface.

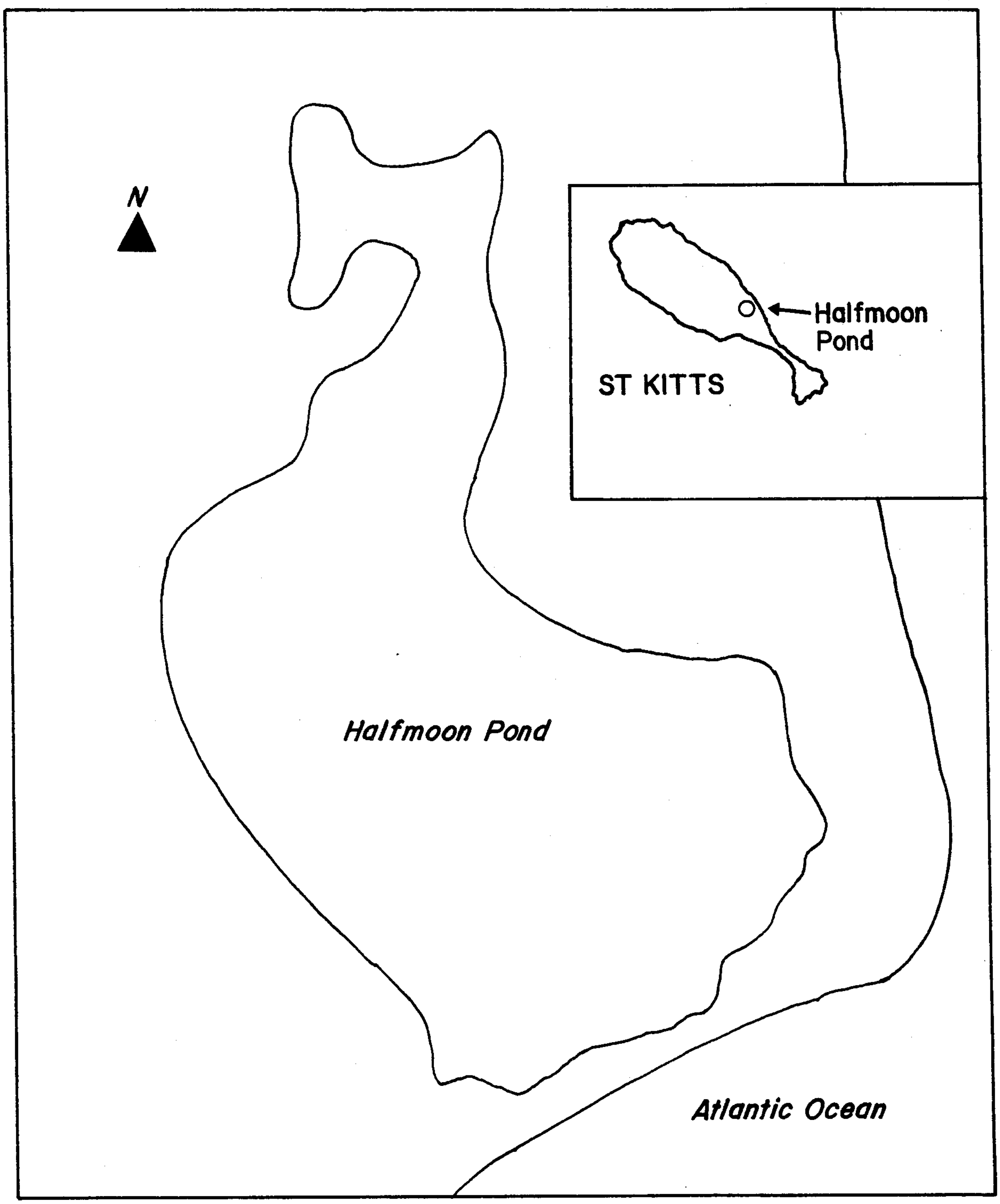
2. Periodic Monitoring

Certain ponds were selected from among those initially surveyed for continued monitoring in order to gain a better understanding of natural fluctuations in Artemia populations. On each study island, at least one pond previously found to contain Artemia was monitored at varying intervals by a local technician. Temperature was measured by mercury thermometer, and salinity by hydrometry (Anonymous, 1980). If present, live Artemia and cysts were collected and stored as described above for subsequent biochemical analysis.

3. Intensive Study

Halfmoon Pond, located on the eastern coast of St. Kitts, W.I. (Figure 8) was selected for intensive study. This pond has a total area of 17 ha, and is currently under development as a commercial penaeid shrimp farm by Leeward

Figure 8 -- Halfmoon Pond, St. Kitts



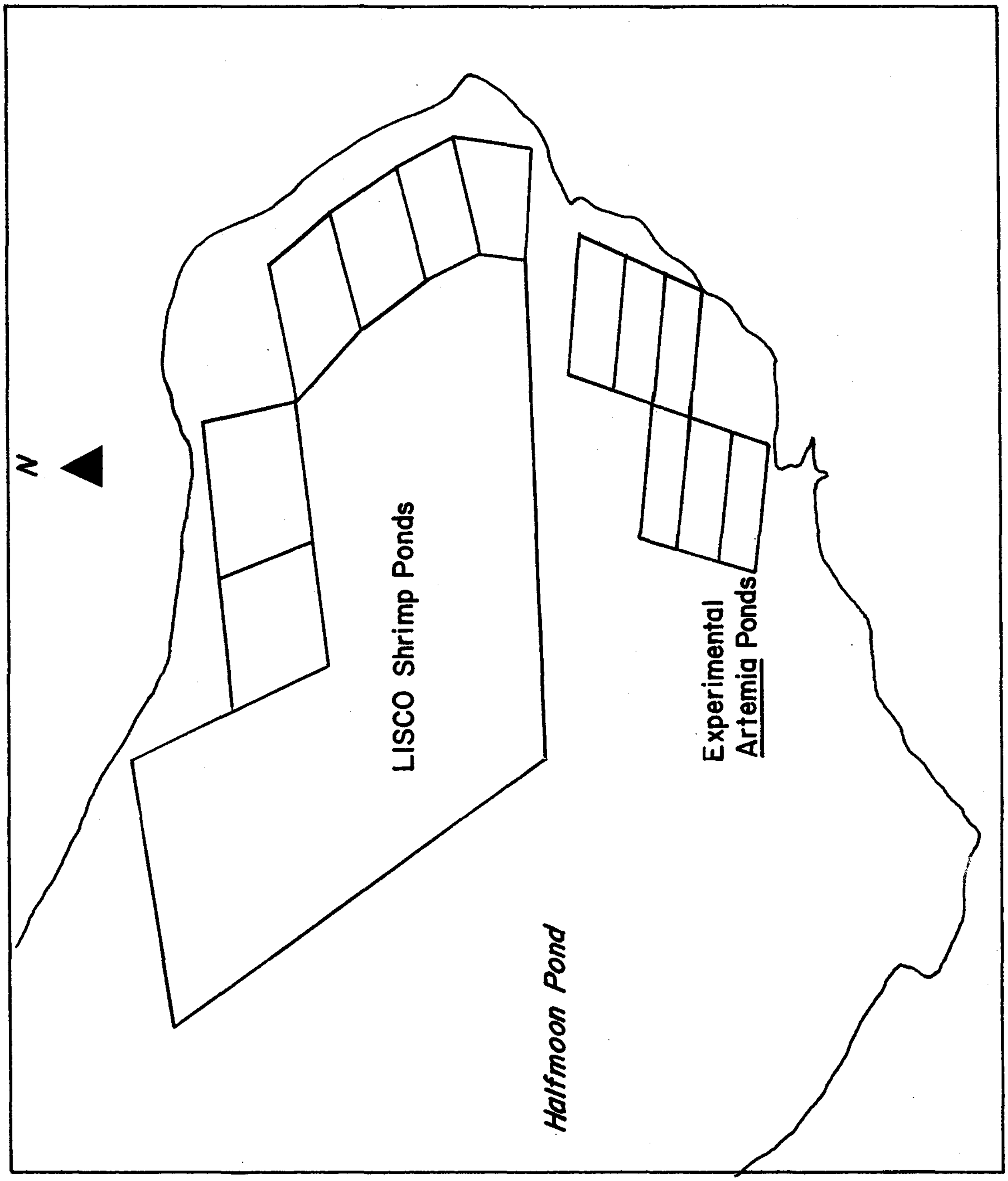
Islands Shrimp Company (LISCO). The on-site availability of laboratory facilities, utilities, and equipment for manipulation of experimental ponds made this an ideal location for the proposed work.

In order to obtain replicate observations of the response of endemic Artemia to natural and artificial changes in local salt ponds, six 0.1 ha experimental ponds were constructed adjacent to the area developed by LISCO (Figure 9). Using a backhoe and manual labor, levees were built by piling sand and mud from the pond bottom to a height of approximately 30 cm above water level. Corrugated plastic drain pipes 15 cm in diameter were embedded in levees adjacent to each pond.

Temperature, salinity, pond depth and weather conditions were regularly monitored in each experimental pond. Initially, measurements were made between 0800-0900, 1100-1300, and 1500-1700 hrs daily. This schedule was subsequently reduced to morning and mid-afternoon periods which were found to give results comparable to the more intensive regime. Temperature and salinity were measured in surface and bottom waters at the four corners of each pond. Salinity was routinely estimated by means of an A-O model 10430 refractometer using samples collected from the desired depth with a pasteur pipet. Twice during the study, readings obtained with the refractometer were compared with salinity values calculated by Mohr titration (Strickland and Parsons, 1972). On both occasions refractometer readings were within 5% of titration values. Temperature was measured with a YSI model 43 electronic thermometer equipped with a thermistor attached to a wooden pole. A graduated dowel was permanently installed in each pond to permit depth estimates. Rainfall was measured with a rain gauge installed on the eastern border of the ponds. Windspeed was estimated by means of a hand-held wind meter (accuracy +/- 5%).

Dissolved oxygen was determined in the experimental ponds at irregular intervals via a modified Winkler procedure (Strickland and Parsons, 1975) using pre-packaged reagents (Hach Chemical Co.). An attempt was made early in the study to use a polarographic oxygen probe (Yellow Springs Instrument Company) for this determination, but interference from high salt concentrations prevented reliable measurements. Water samples for analysis were collected in 300 ml BOD bottles by inverting the bottle, lowering to the desired depth, and venting trapped air to the surface by means of a short length of plastic tubing.

Figure 9 -- Arrangement of Experimental Ponds



Biochemical oxygen demand was estimated over 24 hours (this interval is shorter than the standard BOD determination, but was necessitated by extremely low ambient oxygen concentrations). Water samples were collected as described above, with duplicate samples being collected in bottles which were covered with black vinyl tape so as to exclude light. Dissolved oxygen was immediately determined in the untaped samples. The dark bottles were closed with ground glass stoppers and allowed to incubate at the collection site for 24 hours, after which dissolved oxygen was determined as described above. Comparison of the samples provided an estimate of 24-hour BOD.

pH was measured at irregular intervals, initially with a Hach model 16400 pH meter and later with an Orion model 407A/F specific ion meter. Samples were collected in polyethylene jars and pH measured within 0.5 hr of collection.

Soluble iron has been identified as an important factor in hemoglobin synthesis and cyst production in vitro. Accordingly, this parameter was periodically measured using a Hach model IR-18 test kit (sensitivity = 1mg/l).

Because of probable chemical changes in water samples stored for more than 24 hours, it was desired to perform on-site analyses for reactive phosphorus, nitrate, nitrite, and ammonia. Reactive phosphorus, nitrate, and nitrite were determined according to methods given in Strickland and Parsons (1972). An attempt was made to measure ammonia using the phenate method (Anonymous, 1980), but the reagents precipitated in the hypersaline pond waters (probably due to carbonate interference). Because of this difficulty and levels of nitrate/nitrite which were close to the detection limits of the methods used, it was decided to submit additional samples to a better-equipped analytical laboratory in St. Croix. These samples were stored at -20C (because of high salinity they did not freeze) prior to shipping.

Population biomass and reproductive mode of adult Artemia were the parameters used to evaluate the response of endemic brine shrimp to changes in experimental ponds. Biomass in each pond was estimated on a semi-daily basis. Using reference markers on the levees, each pond was visually divided into 36 sections. On sampling days, ten sections in each pond were selected using a table of random numbers (it was found in preliminary work that increasing the sample size did not materially affect variation between samples, and that ten samples were the maximum which could be accommodated in the overall work schedule). Each section was sampled by lowering a section of 11 cm diameter plastic pipe to the bottom of the pond, and slipping a fiberglass sheet beneath the pipe so that the surface layer of mud was contained within. This "core" sample was seived through a 220 u nylon screen, and Artemia retained on the screen were counted.

Reproductive mode was determined by microscopic examination of oviducts, uteri, and shell glands in adult female brine shrimp. 50 to 100 Artemia were retained during biomass sampling, and were examined individually until 20 adult females had been inspected. The following data were recorded for each sample: total numbers of male adults, female adults, juveniles and nauplii; presence and color of cysts/eggs in oviducts or uteri; color of shell gland, and number of riding pairs (males attached to females, but not necessarily copulating). Early in the study, 16 adult females were isolated to confirm the relationship between shell gland or gamete color and reproductive mode. All Artemia with red shell glands became cyst-bearers and all red gametes became cysts, while all grey gametes became nauplii.

Periodic samples were taken of cysts produced in experimental ponds. Initial efforts were directed toward devising a semi-quantitative procedure which would permit comparison of cyst production among the ponds under study. Collectors were fabricated from 15.2 cm lengths of 7.6 cm (internal diameter) PVC pipe covered at one end with 160 u nylon screen. A sharpened dowel passing through the center of the pipe was used to secure the collector in a half-submerged position in the pond to be sampled. While these devices did collect cysts, wind-driven movement of surface waters tended to partially flush the collectors so that quantitative collection was doubtful. Moreover, the collectors also tended to accumulate suspended organic matter which made it difficult to subsample and count the cysts. The microscopic examination procedure described above appeared to provide a more reliable index of cyst production, and cyst collectors were consequently not used on a continuing basis. Subsequent collections of cysts for biochemical analysis were made by dragging a 202 u dip net at the water surface through the center of each pond.

Collected cysts were cleaned by rinsing with fresh water over 355 u (which passed the cysts) and 106 u (which retained the majority of cysts) sieves. Cysts were then rinsed into separatory funnels with saturated brine, and aerated. After 48 hr, aeration was stopped, and settled debris drawn off. Cysts were rinsed over a 106 u sieve and returned to separatory funnels with distilled water. After brief aeration, cysts which did not float (floating cysts are not normally viable) were drawn off and air dried.

Hatching percentage of selected cysts determined by weighing approximately 0.25 g air-dried cysts into 100 ml graduated cylinders containing 80 ml filtered natural seawater. Gentle aeration and continuous light were provided, and each cylinder was examined at intervals for newly-hatched nauplii. Percentage hatch was determined after 36 hr, by counting settled cysts (floating cysts were disregarded) and nauplii in five 0.25 ml volumes from each cylinder. Each aliquot was treated with a drop of tincture of iodine to immobilize nauplii.

A brief peripheral experiment was conducted to compare the efficacy of St. Kitts and San Francisco Bay *Artemia* as food for penaeid shrimp postlarvae. Cysts collected from the Great Salt Pond in January, 1984 were cleaned by placing approximately 60 g of the cyst mass in a 2 l separatory flask containing 1 l of saturated brine. Aeration was provided for 24 hours to separate cysts from hatching debris. The procedure was then repeated for an additional two hours, after which the cysts were briefly rinsed over a 106 u sieve with tap water. The cysts were returned to a 2 l separatory flask containing tap water and gently aerated at one-half depth for approximately ten minutes during which viable cysts sank and were removed. This procedure yielded 7.7 g cleaned cysts after air drying.

Cysts to be hatched for the feeding experiment were placed in a graduated cylinder containing filtered seawater, and aerated for 21 h at 25 - 30 C under constant fluorescent light. San Francisco Bay cysts ("Living World" batch B0747) were obtained from a pet store in St. Kitts. The latter cysts were rinsed and incubated as described above for 22 hours.

Larvae of *Penaeus vannamei* were hatched from broodstock in the LISCO maturation laboratory and raised on a diet of Tahitian *Isochrysis* and *Tetraselmis* to the first post-larval stage. 31 post-larvae were placed in each of six 1.0 l glass beakers containing filtered sea water. To three of the beakers were added approximately 4,400 *Artemia* larvae hatched from St. Kitts cysts. An equivalent number of larvae hatched from San Francisco Bay cysts was added to the remaining beakers. Gentle aeration was provided, and new *Artemia* nauplii were provided every other day from freshly hatched cysts. Prior to each feeding, penaeids were held briefly in a 450 u nylon mesh bag suspended in sea water while water was exchanged and the beakers cleaned. After 12 days the penaeids in each beaker were counted and individually measured to determine survival and growth rate respectively.

In order to estimate the density of planktonic algae, known volumes of pond water (typically 150 - 600 ml) were filtered through 90 u nylon mesh, then centrifuged in 10 ml aliquots for 15 minutes at approximately 1600G. The resulting pellets were resuspended, pooled, recentrifuged, and finally suspended in 0.5 ml water. Density of algal cells in the concentrated samples was estimated using a hemacytometer.

Complete identification of algal species was not possible due to the absence of taxonomic studies on species endemic to Eastern Caribbean salt ponds. Representative samples of commonly encountered forms were preserved in 10% formalin for later examination by a consulting algologist. Prior to examination, samples were allowed to settle for at least 12 hours, after which an aliquot was pipetted from the bottom of the container for microscopic examination. Cell sizes were determined with an ocular micrometer. Algae generally were identified to at least the division level, but in many cases to the order, family or genus level. In some cases small size of the organisms prevented identification even to the division level. Texts consulted included Fritsch (1965), Butcher (1959), Smith (1951), Fogg *et al*. (1973), and Chapman and Chapman (1973). The taxonomy scheme followed is that given by the latter authors.

Statistical analyses were conducted according to procedures given by Sokal and Rohlf (1981). Data were tested for equality of variances by the F-max test, and for normality by the Kolmogorov-Smirnov test. Percentage data were transformed to arc-sine square roots prior to analysis.

B. Investigations at University of the West Indies

The response of Eastern Caribbean *Artemia* to variations in temperature, salinity, and dissolved oxygen was evaluated at the University of the West Indies (Cave Hill, Barbados).

Because of the scant information available on *Artemia* endemic to the Eastern Caribbean, two preliminary studies were undertaken to evaluate hatchability of *Artemia* cysts collected in St. Kitts, and to select suitable algal diets for rearing brine shrimp hatched from these cysts.

Hatchability studies in media of various salinities were conducted using two collections of cysts obtained from the Great Salt Pond ("GSP"), St. Kitts, one in January, 1983 and the other in September, 1983, and cysts obtained from the Artemia Reference Center ("ARC") (Ghent, Belgium). 0.25 to 2.0 g cysts were placed in 1 l flasks containing seawater adjusted to the desired salinity with distilled water or sodium chloride. Triplicate 1.0 ml aliquots were withdrawn for cyst/larvae counts immediately following the addition of cysts and at daily intervals thereafter.

Algal cultures of _Tetraselmis_ sp., _Nannochloropsis salina_, and _Nannochloris stomus_ were prepared from innocula obtained from the Culture Collection of Marine Phytoplankton (Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Me). Stock cultures were prepared as follows: Autoclaved 10 mm x 150 mm glass tubes containing 10 ml sterile f/2 medium (Guillard and Ryther, 1962) were innoculated with three to six drops of parent algal culture, plugged with cotton wool, and covered with aluminum foil. These cultures were incubated at 25 C under indirect sunlight supplemented with 14 hr/day fluorescent lighting. Aeration was provided by shaking twice daily. When an algal "bloom" was evident in the cultures, 250 ml conical flasks containing 100 ml sterile f/2 medium were innoculated using 5 ml from two culture tubes for each flask. The latter cultures were incubated as described, and were used to innoculate 20 l volumes of f/2 medium. The same light and temperature conditions were employed during incubation of large volume cultures; aeration was constant.

The suitability of these algae and of powdered wheat germ as a diet for _Artemia_ culture was evaluated over a period of 20 days. Larval brine shrimp were hatched from GSP or ARC cysts, and 70 - 120 larvae placed in 1 l flasks containing normal seawater. Minimal cell densities of 70,000 (_Tetraselmis_) or 250,000 (_Nannochloropsis_ and _Nannochloris_) per ml were maintained in flasks used to compare algal diets. Wheat germ was evaluated at 0.15 g per day addition level. Numbers of surviving animals were recorded daily. Four to seven replicate evaluations were made for each diet with each _Artemia_ strain.

Debris was removed by pipetting from the bottom of each culture on a daily basis. This procedure removed about 10% of the medium which was replaced by water of the appropriate salinity. Every third day, approximately one-third of the total volume was removed and replaced with fresh medium. When the medium became particularly cloudy (as was frequently the case with wheat germ-fed cultures), the entire culture volume was replaced. Using plankton netting, brine shrimp were strained from the culture into a small volume of water in a petri dish, from which they were transferred to fresh medium using a wide-bore pipet.

The effects of variations in salinity and temperature on *Artemia* growth and survival were evaluated as follows. For each factor combination to be tested, four 1 l glass beakers were prepared, each containing 0.8 l seawater adjusted to the desired salinity with sodium chloride or synthetic sea salts. Initially, 800 newly-hatched *Artemia* from GSP or ARC cysts were added to each container. In later experiments the number of *Artemia* was reduced to 200 because of the low hatchability of GSP cysts. A diet of *Tetraselmis* sp. at 70,000 cells/ml was used throughout these experiments. Each container was provided with constant aeration. Temperature was maintained with room air conditioning and thermostatically controlled temperature baths. The culture medium was replaced periodically as described above. Observations of survival and maturation state were made daily. Duration of these observations ranged from 4 to 41 days, depending upon survival.

An attempt was made to induce cyst production by salinity stress. 20 10 mm x 150 mm glass culture tubes were prepared, each containing 20 ml hypersaline medium (120 o/oo) and a pair of adult *Artemia*. Salinity in each culture tube was raised in daily increments of 10 o/oo to 150 o/oo over a period of three days. The salinity in ten of the culture tubes was then lowered in daily increments of 30 o/oo to a final salinity of 60 o/oo over a period of three days. Production of larvae and cysts was noted daily.

Reproductive capacity of selected *Artemia* cultures was evaluated by isolating ten riding pairs of adult *Artemia* in culture tubes as described above. These animals were observed for 18 - 35 days, and production of larvae and cysts noted periodically. Feeding and physical factor regimes remained unchanged from the original culture.

C. Biochemical analyses

Samples of live *Artemia* and cysts were collected for biochemical analyses from the ponds discussed above.

Approximately 1 g wet weight of sample was extracted with a methanol:chloroform:water mixture as described by Bligh and Dyer (1959). Saponification and methylation were carried out as described by Schauer et al. (1980). The resultant fatty acid methyl esters were separated on a stainless steel column 1.8 m x 2 mm packed with 10% SP-2330 on 100/120 Chromosorb WAW, column and inlet temperature = 200 C; detector temperature = 250 C; nitrogen gas carrier 27.5 ml/min; detector = FID, sensitivity = 0.0000000004 AFS.

Initially, samples of live Artemia for biochemical analysis were preserved in 5% formalin and shipped to the University of Rhode Island for analysis. Fatty acid profiles of these samples were so unusual that it was decided to extract lipids from whole tissue in the field to reduce the likelihood of sample deterioration.

Isoelectric focussing studies were undertaken to examine genetic relationships between selected Artemia populations. Nauplii from each population were hatched, pooled, and homogenized. These samples were analyzed using polyacrylamide gels on an LKB multiphor isoelectric focussing apparatus in pH ranging from 3 to 10. An ampholyte (Sigma "pharmolyte") was incorporated into the gels. Constant power (25 w) was applied. The fractionated proteins were stained, then scanned with an LKB 2202 laser densitometer.

Analyses of chlorinated hydrocarbons were performed by gas chromatography using standard methods.

D. Investigation of Possible Manipulations to Optimize Artemia Production

Results from preliminary and intensive pond studies suggested two types of manipulation which might improve Artemia production in Eastern Caribbean salt ponds: (1) fertilization to promote the growth of algal species which, when consumed by brine shrimp, would impart a desirable fatty acid composition; and (2) lowered salinity as a means of increasing the proportion of cyst-producers among reproductively active Artemia. These options were studied in conjunction with intensive pond studies described above.

Investigation of fertilization options concentrated on two areas: (1) recognition of endemic algal species which would respond to fertilization in hypersaline waters; and (2) evaluation of locally available waste products as potential fertilizers. An initial screening study was conducted using 10% filter press mud and 5% bagasse (both waste products of local sugar cane processing), 11% chicken manure, 11% brewery grain waste, and 6% "NPK" (a locally available agricultural fertilizer used by LISCO to fertilize ponds containing penaeid shrimp). The indicated concentrations reflect the amount of raw material added to 225 ml pond water which had been previously filtered through 10 u nylon mesh and a sintered glass plate. In all cases, some material remained undissolved; the supernatant of these slurries was used in subsequent manipulations.

Supernatant solutions of each slurry were diluted to concentrations of 1%, 0.1%, and 0.01% (percentage supernatant) with filtered pond water (salinity = 198 o/oo). An algal innoculum was prepared by collecting 500 ml pond water from a location in which Artemia were "swarming" (see section III; this procedure was intended to collect algae which might actually be consumed by Artemia in situ). This volume was filtered through 90 u nylon mesh, and centrifuged as described above. Pellets were combined and re-suspended in a final volume of 12 ml. Triplicate 9.8 ml aliquots of each fertilized medium were combined with 0.2 ml algal innoculum in 100 mm x 16 mm glass culture tubes stoppered with cotton wool. Additional innoculations were made into triplicate aliquots of unfertilized pond water, and pond water which had been enriched to produce the f/2 culture medium described by Guillard and Ryther (1962). Additional culture vessels were prepared with f/2 media having salinities of 94 o/oo and 47 o/oo. Cultures were incubated at ambient temperature under natural sunlight filtered through a translucent fiberglass roofing panel. Algal density in the cultures was determined at intervals using a hemacytometer.

Algae which responded favorably in enriched hypersaline media were maintained in culture by transferring 1 ml volumes from the parent cultures to sterile culture tubes containing 9.0 ml f/2 medium having a salinity of 188 o/oo. Larger-volume cultures were also prepared in sterile 250 ml erlenmeyer flasks containing 150 ml of the same medium. All culture vessels were stoppered with cotton plugs, and incubated as described above.

Because the results of initial studies were equivocal with respect the value of the waste products as fertilizers, a second study was conducted using 10% concentrations of the supernatants described above in media with salinities of 90 o/oo and 188 o/oo (note that these were the same slurries which had been prepared for the earlier study; slurries had been aerated constantly during the intervening time). Filtered pond water diluted with distilled water was used to prepare these media. Media and culture tubes were autoclaved at 15 psi for 20 minutes prior to innoculation. 188 o/oo cultures were innoculated with algae which had grown in f/2 medium in the first study, while 90 o/oo cultues were innoculated with water from experimental pond #5 (which supported the largest biomass of Artemia among the ponds studied). These cultures were incubated as described above.

Samples of undiluted slurries were frozen and forwarded to Worldwide Protein Inc. (St. Croix, USVI) for nutrient analyses. After filtration through 0.45 u glass fiber filters, dissolved nitrate, nitrite and ammonia analyses were performed on a Technicon Autoanalyzer II. Dissolved phosphate was determined by methods described by Strickland and Parsons (1972).

Because it seemed possible that the Artemia under study derived a substantial portion of their nutrition from benthic materials, the response of benthic algae to various fertilizers was of interest. 10 l volumes of water from experimental pond #5 were filtered through a 355 u screen and placed in 12 vinyl tubs (45 cm dia x 20 cm). "Mud" was collected from the bottom surface of the same pond, and a sufficient quantity added to each tub to produce a depth of 1 cm. f/2 concentrate was added to the tubs to provide triplicate cultures enriched at a level of f/4, f/40, and f/400 (these concentrations were selected due to concern with the possibile adverse effects of overenrichment on algae which normally inhabit oligotrophic waters). Tubs were incubated at ambient temperature under a combination of natural and fluorescent light. A second series of glass culture tubes were prepared using 10 ml volumes of 1% concentrations of the waste product supernatants described above. Additional culture tubes were prepared with f/2 medium and a medium containing final concentrations of 0.1% NPK + 0.05% Superphosphate. Media and culture tubes were autoclaved and innoculated with 1 ml mud from pond #5. The latter cultures were incubated at ambient temperature under sunlight filtered through translucent fiberglass roof panels.

Having recognized algae which responded favorably to fertilization in hypersaline media, it was desired to determine whether these algae could serve as foods which would produce favorable fatty acid profiles in endemic Artemia. Cysts which had been collected from the Great Salt Pond (St. Kitts) were soaked for an hour in fresh water, then transferred to a 1 l separatory funnel containing normal seawater. Aeration was provided to keep the cysts in suspension. After 24 hours, six 250 ml erlenmeyer flasks were prepared, each containing 150 ml normal seawater and approximately 385 nauplii which had emerged from the cysts. Volumes of algal stock cultures were added to give a concentration of 60,000 cells/ml in each flask. Three of the flasks received algae cultured from experimental ponds, while the remaining three received a culture of Tahitian *Isochrysis* maintained by LISCO. Salinity of the medium in all vessels was adjusted to 90 o/oo with filtered pond water. Flasks were wrapped with aluminum foil to prevent growth of algae which might have been introduced with the nauplii. Algal cell counts were performed at 8 hour intervals, and sufficient volumes added from the appropriate culture to maintain an algal density of 60,000 cells/ml. After one week, larval and juvenile *Artemia* in each flask were counted, then extracted for fatty acid analysis as described above.

Experimental ponds were used to further investigate the possibility of inducing oviparity *in situ* by sudden reduction of salinity. When the latter investigation was begun, salinity in all of the experimental ponds exceeded 300 o/oo, most had begun producing salt, and no live *Artemia* were evident. It was decided to lower the salinity of all experimental ponds to approximate that of the undisturbed portion of Half Moon Pond ("main pond"), innoculate each experimental pond with live *Artemia* collected from the main pond, then to further reduce the salinity in three of the ponds to test the hypothesis that sudden salinity reduction can trigger oviparity. Normal sea water was pumped into each experimental pond by coupling lengths of 15 cm dia plastic conduit and 7.5 cm dia canvas fire hose onto the electric pumping system used for the LISCO shrimp ponds. Pumping times were initially calculated to introduce sufficient volume so that the salinity of experimental ponds would match the main pond. Normal seawater and hypersaline water in the experimental pond were sharply stratified, and vertical mixing was extremely slow. Salinity profiles were prepared for each pond which suggested that if the ponds were fully mixed the salinity in all cases would have been approximately 150 o/oo, i.e., well below that of the main pond. To compensate for this problem, ponds 2, 3, and 5 were opened to the main pond so that water was exchanged, resulting in salinities of approximately 250 o/oo. Water samples were collected from each pond prior to and immediately following salinity manipulations. These samples were frozen in polyethylene containers and forwarded for nutrient analyses as described above.

Live *Artemia* were collected from the main pond by dip net, and pooled in buckets containing 8 l pond water. Numbers of *Artemia* in each bucket were estimated by counting the number of brine shrimp contained in a 10 ml sample collected from the total volume after manual agitation. Sufficient volumes were added to each experimental pond to provide a stock of 9,500 - 10,000 *Artemia*.

Core samples and broodpouch examinations were performed on alternate days as described above. Initial samples showed that random selection of core sample sites did not provide sufficient numbers of brine shrimp to adequately assess reproductive mode. Because reproductive mode was of primary concern (as opposed to quantitative survey), subsequent samples were collected from visible concentrations of *Artemia*.

III RESULTS

A. General Description of Ponds Surveyed and Endemic *Artemia* Populations

Physiographic and ownership data on ponds surveyed are summarized in Table 1. Physico-chemical and *Artemia* distribution data are presented in Table 2.

1. Anguilla

The geology of Anguilla is coralline; the maximum elevation on the island is 59 m. The island contains ten salt ponds, nine of which were surveyed. Traditional salt harvesting continues at one pond, but has been discontinued at others. The government permits dredging at Maunday's Bay Pond, Cove Pond, and Meads Bay Pond for construction purposes. This is locally regarded as a conservationist policy, since sand was previously mined from beaches. The latter practice has reportedly ceased due to the availability of sand from the ponds.

Road Salt Pond (AX1) has, for several generations, been used for the production of salt. An intake ditch is opened once yearly to allow seawater to enter the northwest corner of the pond, from which salt is harvested by hand. A diked trench encircles the pond to prevent rainwater runoff from entering. Except on the west, the pond is surrounded by bluffs covered by low forest scrub bush grazed by goats. The area is residential, with construction of homes in progress nearby. The pond itself is leased from the government, while surrounding land is entirely private.

West End Salt Pond (AX2) was last used for salt production in 1976, though a rudimentary seawater control system still exists. More recently, an aquaculture project for northern lobster was established at the pond, but is not currently in operation. The south-facing pond is privately owned and is set in flat land surrounded by low forest and scrub. The pond bottom is soft. A dike on the west shore retards infiltration from a freshwater spring.

Maunday's Bay Pond (AX3) is bisected by an incomplete dike. It is government owned, while the land to the south (a narrow strip of waterfront) is leased by a developer, and land to the north is privately owned. Vegetation is brush and low woodland. Fish were present in the pond in December, 1983. The pond has a soft bottom, parts of which dry out periodically. To the north, a hillside slopes from an elevation of 23 m to the pond over

Table 1: Physiographic Data and Ownership of Ponds Surveyed

Pond Code	Location	Size (ha)	Depth (1)	Rain (2)	Evap. (3)	Berm (4)	Sea (5)	Own (6)	Comments
<u>Anguilla</u>									
AX1	Road Pond	45	1	<75	N	2	100	V	salina
AX2	West End Pond	18	<1	<75	N	3	100	V	old salina
AX3	Maunday's Pond	14	ND	<75	N	2	75	V/B	
AX4	Meads Bay Pond	20	>1	<75	N	10	200	B	sand mining
AX5	Cove Bay Pond	75	>5	<75	-	3	50	V/B	sand mining
AX6	Mt. Fortune Pond	---	--	--	-	-	---	-	being filled
AX7	Savannah Bay Pond	20	0.7	<75	N	4	150	V	
AX8	Long Salt Pond	23	<0.5	<75	Y	1	100	V	old salina
AX9	Rendezvous Pond	25	0.4	<75	Y	2	100	V	
<u>Antigua</u>									
AN1	McKinnon's Pond	40	0.5	105	Y	0.5	20	V	marina planned
AN2	Cocks Hill Pond	10	ND	120	-	2	300	V/B	dump site
AN3	Jolly Hill Pond	100	<0.1	120	Y	-	-	V/B	swamp
AN4	Deep Bay Pond	18	1-2	120	N	2	60	V	being dredged
AN5	York's Pond	13	ND	120	N	1	-	V	open to sea
AN6	Pinching Bay Pond	12	ND	120	N	3	-	V	open to sea
AN7	Galley Bay Pond	8	ND	120	N	1	60	V	

1 -- at center of pond (m)
2 -- average annual rainfall (cm)
3 -- Y = greater than 75%; N = less than 75%
4 -- lowest berm height (m)
5 -- distance from sea at closest point (m)
6 -- ownership: V = private; B = public

Table 1 (continued)

Pond Code	Location	Size (ha)	Depth (1)	Rain (2)	Evap. (3)	Berm (4)	Sea (5)	Own (6)	Comments
Virgin Gorda									
BV1	Pond Bay	2	0.7	<100	Y	3-4	100	B/V	
Prickly Pear									
BV2	Brandy Point	2	0.8	<75	N	0.5	20	B	uninhabited cay
BV3	Biras Creek	ND	0.75	<100	N	-	-	V	open to sea
Tortola									
BV4	Pockwood Pond	ND	-	-	-	-	-	-	overgrown
BV5	Belmont Pond	7	0.5	<100	N	4	75	V	
BV6	Cane Garden Pond	3	ND	110	N	2	20	V	waste dump
BV7	Josia's Bay Pond	8	ND	110	Y	1.3	250	V	open to sea
Beef Island									
BV8	Goose Hole Pond	14	ND	<75	Y	0.6	30	V	open to sea
BV9	Bluff Bay Pond	7	ND	<75	-	-	60	V	sand mining
BV10	Trellis Bay Pond	2	0.3	<75	Y	1	60	V	
BV11	Sprat Point Pond	4	>1	<75	N	1-2	50	V	protected
BV12	Conch Bay Pond	4	0.3	<75	Y	1-2	40	B	
Salt Island									
BV13	Large Pond	9	1.6	<75	Y	2	100	B	salt production
BV14	Small Pond	6	0.7	<75	Y	2	50	B	joined to BV13

1 -- at center of pond (m)
2 -- average annual rainfall (cm)
3 -- Y = greater than 75%; N = less than 75%
4 -- lowest berm height (m)
5 -- distance from sea at closest point (m)
6 -- ownership: V = private; B = public

Table 1 (continued)

Pond Code	Location	Size (ha)	Depth (1)	Rain (2)	Evap. (3)	Berm (4)	Sea (5)	Own (6)	Comments
Anegada									
BV16	East End Pond	60	0.2	<100	N	1	60	V/B	part open to sea
BV17	Flamingo Pond	225	0.3	<100	N	1-2	200	V/B	open to sea
BV18	Bones Bight Pond	25	0.75	<100	N	4-5	125	V	open to sea
BV19	Red Pond	150	<1	<100	N	3	100	B	open to sea
St. Kitts									
SK1	Half Moon Pond	17	0.5	<100	N	3	30	V	shrimp farm
SK2	Greatheed's Pond	---------- could not be sampled ----------							
SK3	Frigate Bay Pond	6	1	<100	N	1.5	20	V	marina planned
SK4	Little Salt Pond	27	0.6	<100	-	1-2	150	V	old salina
SK5	Great Salt Pond	203	1.2	<100	N	1-2	600	V	old salina
SK6	Unnamed pond	0.2	0.2	<100	-	1-2	550	V	old salina

1 -- at center of pond (m)
2 -- average annual rainfall (cm)
3 -- Y = greater than 75%; N = less than 75%
4 -- lowest berm height (m)
5 -- distance from sea at closest point (m)
6 -- ownership: V = private; B = public

Table 2: Physico-chemical and *Artemia* Distribution in Ponds Surveyed

Pond Code	Location	Date Sampled	Cysts	Adults	Salinity	Temp	pH
Anguilla							
AX1	Road Pond	17/12/83	-	+	124	27	ND
AX2	West End Pond	17/12/83	+	-	134-138	31.4	ND
AX3	Maunday's Pond	17/12/83	-	-	64	30.1	ND
AX4	Meads Bay Pond	17/12/83	-	+	100-116	30.5	ND
AX5	Cove Bay Pond	17/12/83	-	-	125-130	29.5	ND
AX6	Mt. Fortune Pond	18/12/83	-	-	8	--	ND
AX7	Savannah Bay Pond	18/12/83	-	-	81-87	26.5	ND
AX8	Long Salt Pond	18/12/83	-	+	110-112	28.0	ND
AX9	Rendezvous Pond	18/12/83	-	-	131	30.1	ND
Antigua							
AN1	McKennon's Pond	8/12/83	-	-	53	31.6	ND
AN2	Cocks Hill Pond	8/12/83	-	-	53	31.0	ND
AN3	Jolly Hill Pond	8/12/83	-	-	--	--	ND
AN4	Deep Bay Pond	8/12/83	-	+	236	30.8	ND
AN5	York's Pond	9/12/83	-	-	36	30.9	ND
AN6	Pinching Bay Pond	9/12/83	-	-	36	--	ND
AN7	Galley Bay Pond	9/12/83	-	-	52	30.1	ND
Virgin Gorda							
BV1	Pond Bay	7/11/83	-	-	12	32.4	ND
Prickly Pear							
BV2	Brandy Point	8/11/83	-	-	9	29.2	7.8
BV3	Biras Creek	8/11/83	-	-	5-27	29.2	ND

Table 2 (continued)

Pond Code	Location	Date Sampled	Cysts	Adults	Salinity (o/oo)	Temp (C)	pH
Tortola							
BV4	Pockwood Pond	12/11/83	-	-	--	--	--
BV5	Belmont Pond	12/11/83	-	-	45	31	8.0
BV6	Cane Garden Pond	12/11/83	-	-	12	34	7.4
BV7	Josia's Bay Pond	12/11/83	-	-	7	34	8.4
Beef Island							
BV8	Goose Hole Pond	13/11/83	-	-	37	31	8.3-8.6
BV9	Bluff Bay Pond	13/11/83	-	-	20	ND	8.4
BV10	Trellis Bay Pond	13/11/83	-	-	21	ND	8.0-8.4
BV11	Sprat Point Pond	13/11/83	-	-	ND	ND	7.2-8.4
BV12	Conch Bay Pond	13/11/83	-	+	22-26	28	8.8
Salt Island							
BV13	Large Pond	14/11/83	+	+	89	34.5	ND
BV14	Small Pond	14/11/83	-	+	91	36.1	ND
Anegada							
BV16	East End Pond	7/1/84	-	-	26	ND	ND
BV17	Flamingo Pond	8/1/84	-	-	55	28.4	ND
BV18	Bones Bight Pond	8/1/84	-	-	55	28.1	ND
BV19	Red Pond	8/1/84	-	-	87	32.5	ND
St. Kitts							
SK1	Half Moon Pond	25/11/83	+	+	184	30.1	7.7
SK2	Greatheed's Pond	---------------- could not be sampled ----------------					
SK3	Frigate Bay Pond	26/11/83	-	-	52	28.8	7.6
SK4	Little Salt Pond	27/11/83	-	-	72	31.8	ND
SK5	Great Salt Pond	27/11/83	+	-	230	31.2	7.2
SK6	Unnamed pond	27/11/83	-	-	72	29.6	8.4

a distance of approximately 640 m, and probably introduces some runoff during heavy rains.

Cove Pond (AX5) is part of the same pond system as Maunday's Bay Pond, and shares the same topographical setting. North of the pond is low woodland and scrub, while sand and grassland surround the pond on the remaining sides. The pond and the land to the north are government property, while resorts are being developed to the east and southwest. Sand is dredged from the pond, which is now over 4 m deep. It is viewed by the government as an appropriate site for a marina.

Mead's Bay Pond (AX4) is bordered, except to the north, by low scrub or barren land. The pond is government-owned, but is surrounded by private property. Sand is currently dredged for resort construction. The bottom of the pond is soft in the western end where runoff from surrounding hills enters through a culvert.

Savannah Bay Pond (AX7) is bordered to the west by a small hotel and a watershed covered with low bush, and to the south by private grazing land. This area of the island is sparsely settled at present and somewhat desolate in appearance. The pond bottom is soft, and the water appeared turbid in December, 1983.

Long Salt Pond (AX8) was the site of a shrimp culture project until mid-1983. Earthen raceways and cement tanks remain, with _Artemia_ in the raceways. The berm between the pond and Long Pond Bay immediately to the east is quite low and was previously opened for salt production purposes. The pond and surrounding land are all privately owned. To the west of the pond lies flat, marshy land with low bush and some mangroves; to the south, dunes covered with dense bush. A watershed which feeds the pond from the north is covered with brush and low trees.

Rendezvous Salt Pond (AX 9) was once leased from the government for salt production. Across the southern berm of the pond is a popular beach. All surrounding property is privately owned flat land covered with brush. Residential construction is taking place to the north. The pond, which has a soft bottom, dries out periodically.

2. Antigua

Antigua has a varied terrain and significant tourist industry. Development activity related to the latter shares coastal land with endemic salt ponds, all of which are on the lee side of the island. Of the seven ponds visited, five abut hotels or are being converted into marinas. One is partially used as a dump, and one appears destined to be filled. The salt pond east of Five Islands Village indicated on D.O.S. topographic maps no longer exists, having been filled. Elderly residents remember "picking salt" at Deep Bay Salt Pond and McKinnon's Salt Pond, but this practice seems to have ended many years ago.

McKinnon's Salt Pond (AN1) is situated in the center of a well-developed area just north of St. John's. As of December, 1983, it had been committed by the government for development of a marina. To the east of the pond lies the West Indies Oil Refinery which has in past years contributed to pond pollution (Towle and McEachern, 1974). To the west are numerous small resorts; a larger resort complex abuts the pond on the north. The surrounding flat land is privately held. White and black mangroves border the pond to the south and west; otherwise vegetation consists of grass and scrub bush. The pond essentially dried out during the drought of August, 1984, but this happens infrequently. A low berm to the northwest is occasionally broached by high seas. Fish were present in the pond in December, 1983.

Cocks Hill Pond (AN2) and Jolly Hill Salt Pond (AN3) lie in a swampy low area fed by runoff from surrounding hills and the upland village of Jennings. Cocks Hill Pond is partially filled by a solid waste dump, and appeared very turbid in December 1983. Jolly Hill Pond dries out completely during periods of scant rainfall, and is likely to be filled by the development company which owns it. Surrounding grassland is grazed by cattle.

Deep Bay Salt Pond (AN4) and the surrounding land are being developed into a marina and resort area. In the winter of 1983, a boat channel was dredged to the beach north of the pond. As of August, 1984 the channel had not been opened through to the beach. The pond is surrounded by low hills which are covered with grass, bush, and low forest. The beach at Deep Bay is popular with bathers, and the bay is often used as an anchorage. The pond itself does not dry out and appears to support Artemia throughout the year. It is one meter deep at its center, and the bottom is soft throughout.

Yorks Salt Pond (AN5) is no longer isolated from the sea, a channel being maintained by the residents of a nearby leper colony. Fish now abound in the seawater pond, which stands amidst low forest and mangrove stands.

Pinching Bay Pond (AN6) receives runoff from the hillside to the north, which is covered with scrub and low forest used to make charcoal. The pond bottom is covered by a light algal mat over deep anaerobic mud. The pond and all surrounding property are part of a large private estate. On the southeastern end of the pond, the mangrove-covered berm is open to the sea; the pond is only slightly hypersaline at its western end.

Galley Bay Pond (AN7) lies in a woodland area and is surrounded by dense black mangrove stands. Hillsides drain into the pond from the northeast and southwest. A resort hotel abuts the pond on the northwest and all surrounding land is privately owned. The pond is turbid, with a soft bottom and numerous wading birds.

3. British Virgin Islands

Rainfall was 16.5 cm on Tortola during the week prior to initial site visits in the B.V.I. (Dept. of Public Works, Tortola). Local residents on Virgin Gorda, Prickly Pear, and Salt Island confirmed that rain had also fallen on these islands during the same period, but no measurements had been made.

Virgin Gorda, the second largest of the B.V.I., contains three salt ponds, two of which were surveyed. A town is located on the arid southern peninsula and several toursit resorts, isolated from the remainder of the island except by boat, occupy the east end. There is a mountain (414 m) at the center of the island. Recently, two salt ponds at St. Thomas Bay and Biras Creek have been opened to the sea and dredged or partly filled for tourist development.

Pond Bay Salt Pond (BV1), on the east side of Virgin Gorda, is surrounded on three sides by watersheds. Red and black mangroves border the pond on the south and west. A steep direct road, deeply eroded, leads to the southeast shore. A berm, three to four meters in height, comprises the pond's western border. The government of the B.V.I. leases the surrounding land to a resort corporation which reportedly is inclined to allow the pond to remain in its undeveloped state. The area is uninhabited.

Bandy Point Pond (BV2) is one of two small ponds (less than one hectare) on the south shore of Prickly Pear Cay. Approximately 90% of the pond shore is bordered by white and black mangroves. A few heron and ducks use the pond, and numerous small crabs are present in the shallower areas.

Birás Creek (BV3), on the eastern shore of Virgin Gorda, is privately owned by a resort. It is oriented on a northeast-southwest axis, with the southwest portion apparently having been partially filled by the owners. Approximately one-third to one-half of the southwest portion of the existing pond contains stands of dead mangroves. In November, 1983, there were fish in the pond, although water turbidity prevented identification. The pond bottom is black mud which smells slightly sulfurous when disturbed.

Tortola contains six salt ponds, four of which were surveyed. It has numerous steep watersheds, little flat land, and a maximum elevation of 521 m. As developmental pressure has increased in recent years, the government has authorized considerable dredging and filling of coastal areas.

Pockwood Pond (BV4), though identified as a coastal pond on D.O.S. topographic maps, no longer exists. Red mangroves and other vegetation now cover the entire area, and standing water is virtually absent.

Belmont Pond (BV5), which faces the sea on the west, is near a private residential development and is bordered entirely by dense bush and mangroves. During the rainy season, standing pond water innundates the mangrove roots, while in the dry season water may recede by as much as 20 m on the western shore. The pond bottom is soft to the west, and somewhat firmer on the east.

Cane Garden Pond (BV6), which faces west, is closely abutted by homes and is girded by a road. The property owners have asked the government for permission to fill the pond, indicating an objectionable odor and insect breeding problems. Malfunctioning septic systems allow waste material to enter the pond. It is bordered by mangroves, and the eastern third is a swamp during the rainy season.

Josiah's Bay Pond (BV7), facing west, is completely surrounded by black and white mangroves. It is low in salinity and supports a more lush flora than most other ponds. Avian life is present, and the swampy surrounding land, privately held, is frequently used for grazing cattle, sheep and donkeys. An open channel has been dug between the northwest corner of the pond and the sea to allow for partial drainage during periods of substantial rainfall.

Beef Island is connected to Tortola by a swing bridge. It contains five salt ponds, all of which were surveyed. It is the site of Tortola's airport, with some residential development. Because of the island's windward position and their location on the flat west end of the island, the salt ponds on Beef Island do not receive as much rainfall runoff as those on Tortola.

Goose Hole Pond (BV8), south facing, is opened to the sea by a culvert which runs under a bordering road. This culvert shoals periodically, closing the conduit and causing the pond to disappear during dry periods. Mangroves border the pond except along its south shore. The pond bottom is soft. The government is considering purchase of the pond in order to preserve its role as an open space.

Bluff Bay Pond (BV9), surrounded by mangroves, is on private land on the south side of the island. Its bottom is soft and rainfall enters at its western end from a gently sloping hillside. The pond area is used for the mining of sand for developmental purposes.

Trellis Bay Pond (BV10) dries out seasonally, and appears to receive little runoff from rainfall. With a hard sand bottom and mangrove stands within the pond, it is in fact little more than a shallow depression in a low-lying plain, with water no deeper than 15 cm.

Sprat Point Pond (Banana Wharf Pond; BV11) holds standing water year round. Situated at the foot of a 224 m hill, its water is apparently richer in nutrients as the pond is green and turbid. A strong sulfurous smell is present. Although on private land, development of the pond is unlikely due to its remote location and the presence of unusual plant species which result in government protection. It is virtually inaccessible except by sea due to dense surrounding woodland.

Conch Bay Pond (BV12), facing north, is next to the airport runway between Conch Bay and Long Bay. Its north and west shores are bordered by black mangroves; south is deep grass planted by the airport landscapers. The pond bottom is firm in the western area, soft elsewhere. The beach north of the pond is a popular recreation spot for Tortolans. The pond has traditionally been used for salt harvesting by local residents. Illustrative of the ephemeral nature of _Artemia_ populations, brine shrimp were present in moderate numbers near the pond's west shore on 13 November, 1983. On the morning of 14 November, repeated passes with a plankton net revealed virtually no _Artemia_.

Salt Island lies to the south of Tortola. It is arid and relatively devoid of trees, and has no potable water. Several permanent residents mine salt from the larger pond, a practice which has persisted since colonial days. Annually, the residents of Salt Island collect (by hand) four bags of salt to be delivered to the Queen of England as rent for their continued residence on the island, which is Crown land.

Salt Pond (BV13) is classically thallasohaline; a seawater spring feeds the pond continuously from its northwestern corner. It does not dry out completely under normal seasonal conditions, but salinity rises past the point of saturation during the dry season when salt is taken. *Artemia*, known locally as "pond fry", were present in great numbers during the rainy season of 1983; relatively large quantities of cysts were found ashore during the subsequent dry season. The pond bottom is soft, and local residents report soft mud in the center of the pond to be more than 1.5 m deep. The water line at the shallower western shore of the pond receded more than 20 m between November, 1983 and July, 1984.

Anegada is a low coralline island surrounded by coral reefs and sparsely populated. Anegada supports a minimal tourist trade, while traditional commercial fishing continues on a small scale. While four salt ponds are described below, there are in fact only two distinct salt pond systems; one at the east end, the other a large complex of connected ponds in the center of the island. Numerous investigations of these two ponds have demonstrated salinity gradients which affect the suitability of the ponds as *Artemia* habitats. Both pond systems ultimately open to the sea; central ponds frequently contain fish which are trapped by local net fishermen.

East End Salt Pond (BV15) is very shallow over soft bottom. The pond's western boundary is ill-defined without a distinct beach area. Red mangroves extend well into the pond along the south, west, and east boundaries, while limestone pavement lines the north shore. Farther east is mud 15 - 30 cm deep over loose carbonate sediment. The land north of the pond is government owned, while land to the south and east is privately held. Cattle frequently graze the land around the pond.

Flamingo Pond, Bones Bight Pond, and Red Pond (BV16, BV17, BV18) together comprise the largest pond in the survey area. These ponds are bordered by red, black, and white mangroves which represent perhaps the most lush stand of vegetation on Anegada. Land north of the ponds is privately owned, while that south of the ponds is government property. The ponds themselves are government-owned.

In Flamingo Pond the benthos varies between soft organic material over loose carbonate sand and bare limestone pavement. Large patches of benthic algae are found attached to the bottom or floating in mats on leeward shores. The water in Flamingo Pond is quite clear. A partially separate pond (Vagabond Pond) adjacent to the western portion of Flamingo Pond is more turbid. Vagabond Pond reportedly dries up during portions of the year.

Bones Bight Pond, between Flamingo and Red Ponds, appears very similar to Flamingo, with clear water, numerous juvenile fish, and bush growing virtually to the water's edge.

Red Pond is so named because of its red-brown color. The water is very turbid (approximately 10 cm secchi depth). While Artemia have been reported in the central ponds (Anegada Corporation, Ltd., 1974), none were found in January, 1984.

4. St. Kitts

St. Kitts (St. Christopher) is a volcanic island with an isolated southern peninsula joined to the rest of the island by a flat isthmus. There are nine salt ponds on the island, six of which were surveyed. SK1, SK2, SK3, Major's Bay, and Cockleshell Ponds are situated on the windward wide of the island, placing them at greater risk during storms. Unlike most other islands surveyed, tourism has only recently begun to play a major role in the Kittitian economy. The land is devoted almost entirely to the culture of sugar cane.

Half Moon Pond (SK1) contains empoundments which are used in a commercial shrimp culture project. This is no longer a closed salt pond, since the project involves pumping raw seawater into the empoundments which are enriched with inorganic fertilizers. Artemia are naturally present in the pond, which does not normally dry up. To the west the pond is bordered by a 158 m hill, and to the north by coconut stands and sugar cane fields. The berm is covered by sea grapes, coconut palms and low bush. The pond is privately owned and leased to the shrimp farm company.

Greatheeds Pond (SK2) is surrounded by mangroves and more distally by sugar cane fields. Palm trees are found along the berm. The southern border abuts the island's main dump, the proximity of which probably has a significant effect on the ecology of the pond. Some rainfall runoff may reach the pond from Upper Canada Estate, which is a 343 m (peak) range of hills to the west of the pond. However, much of the runoff is probably diverted north of the pond by ravines.

Frigate Bay Salt Pond (SK3) is enclosed by privately owned development property. To the south a popular beach with minimal vegetation borders the pond, while mud flats and a parking lot abut on the west. Scrub bush borders the east pond. A hotel, whether by design or equipment malfunction, discharges waste into the pond. The resultant odor is periodically quite noticeable. Plans to dredge the salt pond for construction of a marina have not yet been carried out. A drainage culvert has been cut to the sea, resulting in considerable flushing.

Great Salt Pond and Little Salt Pond (SK4 and SK5) are part of an archaic solar salt production facility which is nearest the sea on the western side of the southern peninsula. A freshwater or brackish water spring appears to feed Little Salt Pond, which is nearer the sea; a dike exists to control the seepage of this water into the Great Salt Pond.

In the Little Salt Pond bottom materials are loosely consolidated sand and algal crust over black anaerobic mud. The southwestern shore is mangrove, while mud flats border on the east. A small unnamed and apparently man-made pond (SK6) is between Little and Great Salt Pond.

The Great Salt Pond is the second largest hypersaline pond within the study area. It is virtually surrounded by hills, the highest of which is 319 m. While no official rainfall data are kept for this area, the generally xerophytic nature of the local flora suggests low precipitation (Harris, 1963); comments of local residents support this conclusion. The surrounding topography makes it likely, however, that significant freshwater input occurs through runoff during rainy seasons. Mangroves are found on the northeastern shore, while other shoreline areas are covered with low bush. The bottom of the west pond is firm and lacks a substantial algal mat.

<u>Artemia</u> cysts in relatively high numbers were found ashore in November, 1983, and were harvested on previous occasions by the shrimp farmers.

B. Periodic Monitoring

Physical/chemical and *Artemia* distribution data presented in Table 3.

C. Intensive Study

Mean daily temperature and salinity data are presented in Figures 10 - 15 and 16 - 21 respectively. Salinity increased progressively during the study period as the water in the pond evaporated, though precipitous drops in salinity occured after periods of heavy rainfall. It should be noted that these effects were less marked in the main pond outside the diked area, due to much greater influx of fresh water from the surrounding watershed which tended to offset the effects of evaporation.

Dissolved oxygen, pH, and biochemical oxygen demand data are summarized in Table 4, 5, and 6. Dissolved oxygen did not exceed 4.5 ppm, and was usually below 2.0 ppm during the period of investigation. There was no consistent increase in dissolved oxygen during daylight hours, and morning oxygen concentrations were not regularly dissimilar to those of late afternoon, suggesting that oxygenation processes in the ponds just equal daily consumption, and that photosynthesis plays a minor role in oxygenation of these ponds. Biochemical oxygen demand ranged from 0 to 1.8 ppm/day. pH varied little, ranging from 7.4 to 7.8.

Nutrient and iron analyses are summarized in Table 7. The data confirm that the experimental ponds are oligotrophic, but tend to have higher nutrient levels than normal seawater, probably because of concentration by evaporation.

Population size and reproductive mode data are presented for each pond along with mean daily salinity values in Figures 16 - 21. Differences between ponds are striking, particularly in view of the fact that they were isolated from a common body of water within two weeks. Maximum percent cyst bearers ranged from 23 - 100%; during a period in which salinity ranged from 138 - 180 o/oo. At salinities between 180 and 230 o/oo, the proportion of female *Artemia* bearing live offspring increased. Above 230 o/oo non-reproductive females tended to dominate the samples. At lower (138 - 180 o/oo) salinities, a sudden salinity decrease caused by heavy rainfall was often followed by an increase in cyst production within two to four days.

Table 3: Physical/chemical and *Artemia* Distribution Data in Periodically Monitored Ponds

Location	Date	Salinity (o/oo) (3)	Temperature (C)	A (1)	C (1,2)
Salt Island, BV 13					
	20/2/84	248		+	+
	29/2/84	284		+	+
	7/3/84	291		+	+
	1/4/84	322	40	-	-
	19/7/84	>285	35	+	+
	11/8/84	251	37	-	+
	21/8/84	258	36	+	+
	1/9/84	224		+	+
Beef Island, BV12					
	10/6/84	135	34	-	-
	20/6/84	63	25	-	-
	13/8/84	78	28	-	-
	24/8/84	117	28	-	-
Anguilla, AX1					
	3/5/84	218		+	+
	8/5/84	178	29	+	+
	24/5/84	178	49	+	-
	8/7/84	138	34	+	-
	29/7/84	138	32	+	+
	12/8/84	149	32	+	-
Anguilla, AX2					
	4/5/84	218	30	+	+
	24/5/84	218	30	+	+
	7/7/84	128	33	+	+
	28/7/84	140	30	+	+
	11/8/84	118	33	+	+
Antigua, AN1					
	7/5/84	76	28	-	-
	16/5/84	95	32	-	-
	6/6/84	80	ND	-	-
Antigua, AN4					
	1/5/84	130	29	-	-
	7/5/84	128	29	+	-
	6/6/84	118	29	+	+
	18/6/84	ND	29	+	+
	9/7/84	ND	ND	+	-
	17/7/84	100	ND	+	+

(1) -- A = adults; C = cysts; + = present; - = absent
(2) -- cysts marked + only if found in water
(3) -- salinity values converted to o/oo from degrees Baume based on calibration data obtained in St. Kitts

Figure 10 -- Mean Daily Temperature in Experimental Pond 1

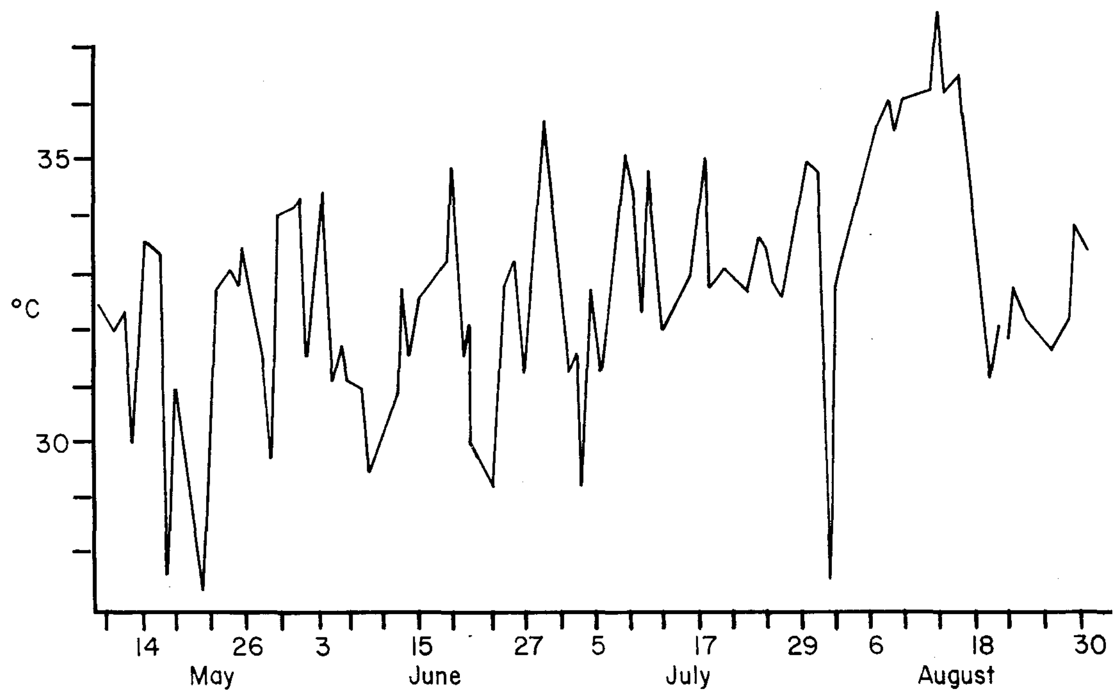


Figure 11 -- Mean Daily Temperature in Experimental Pond 2

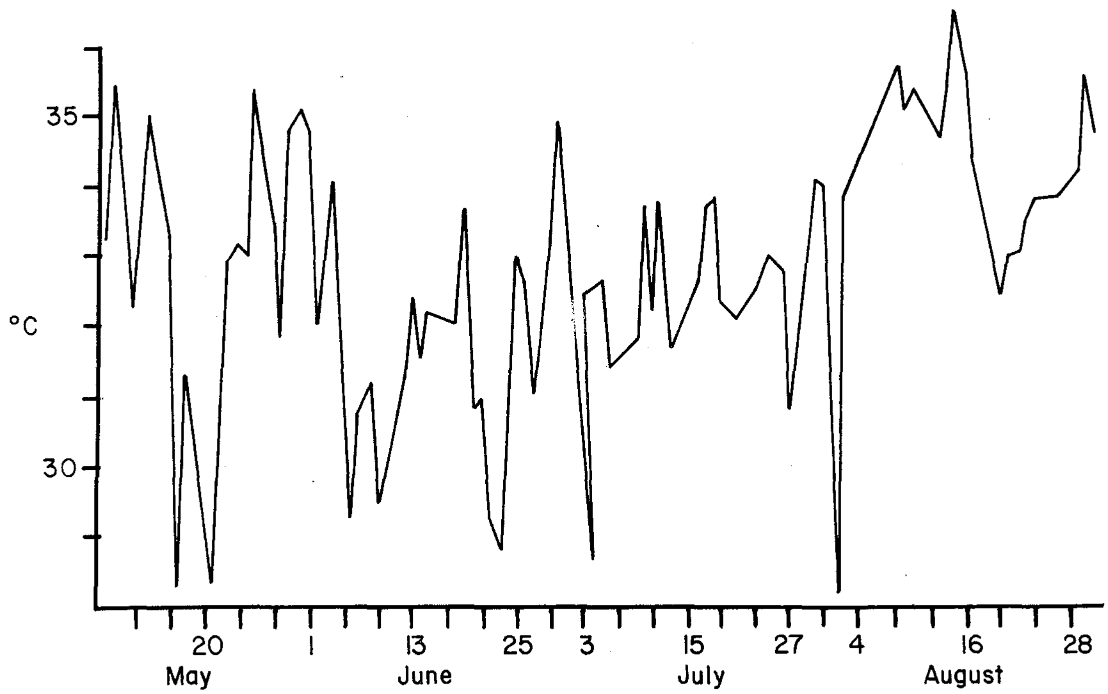


Figure 12 -- Mean Daily Temperature in Experimental Pond 3

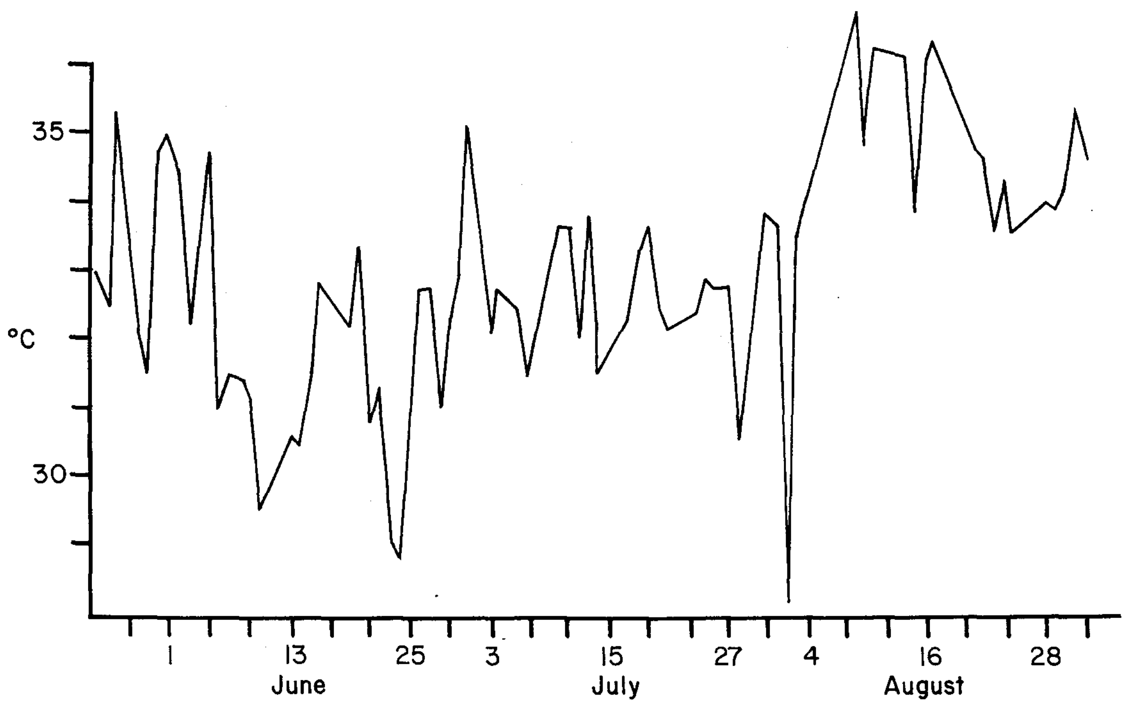


Figure 13 -- Mean Daily Temperature in Experimental Pond 4

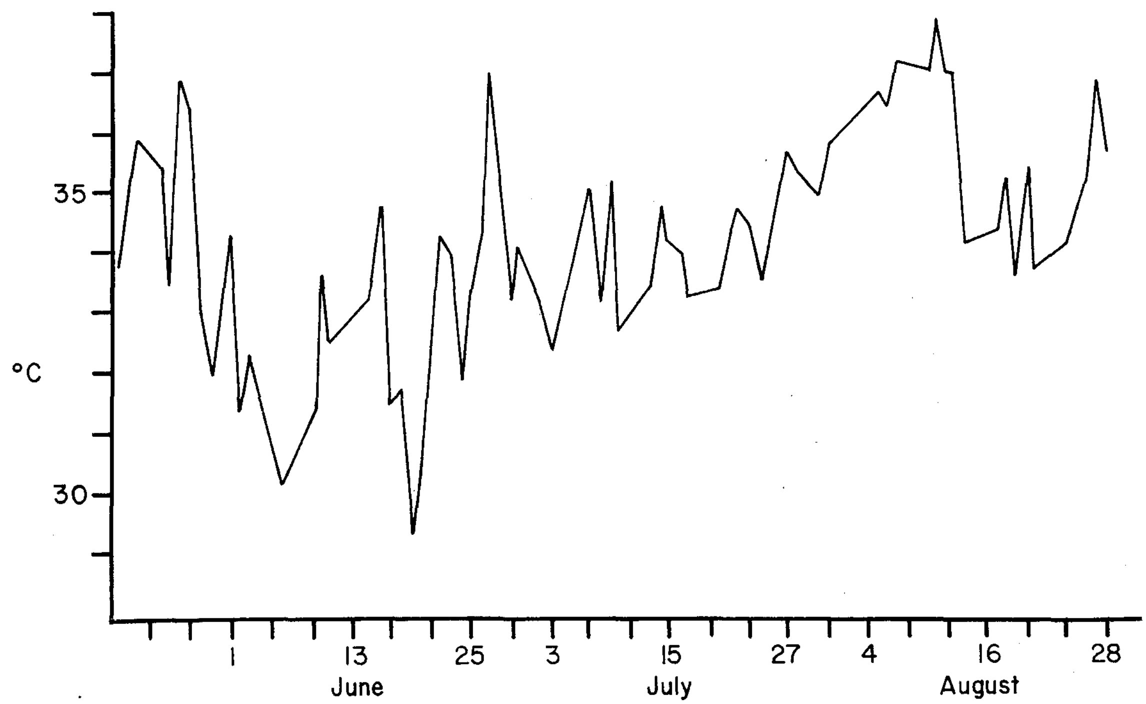


Figure 14 -- Mean Daily Temperature in Experimental Pond 5

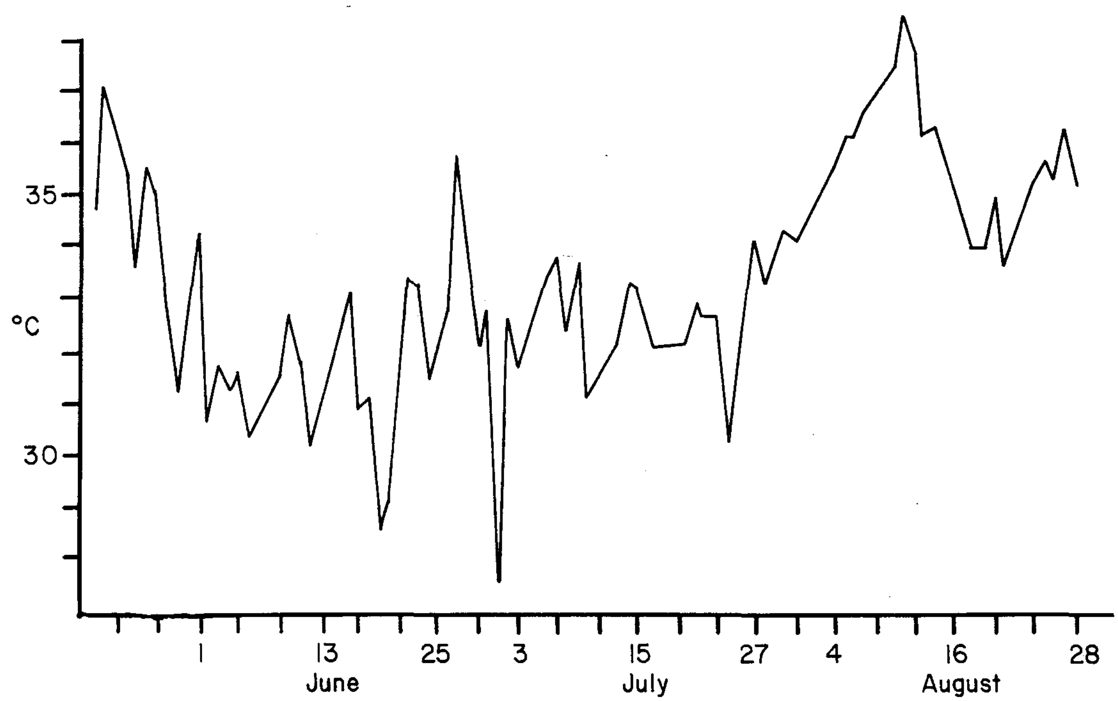


Figure 15 -- Mean Daily Temperature in Experimental Pond 6

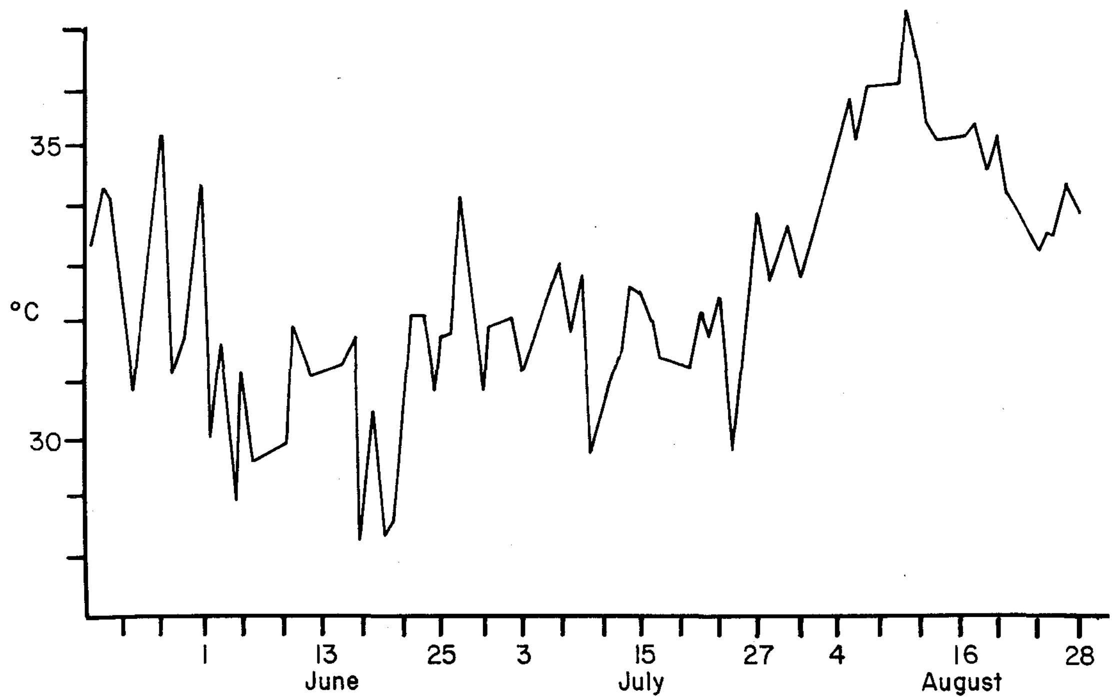
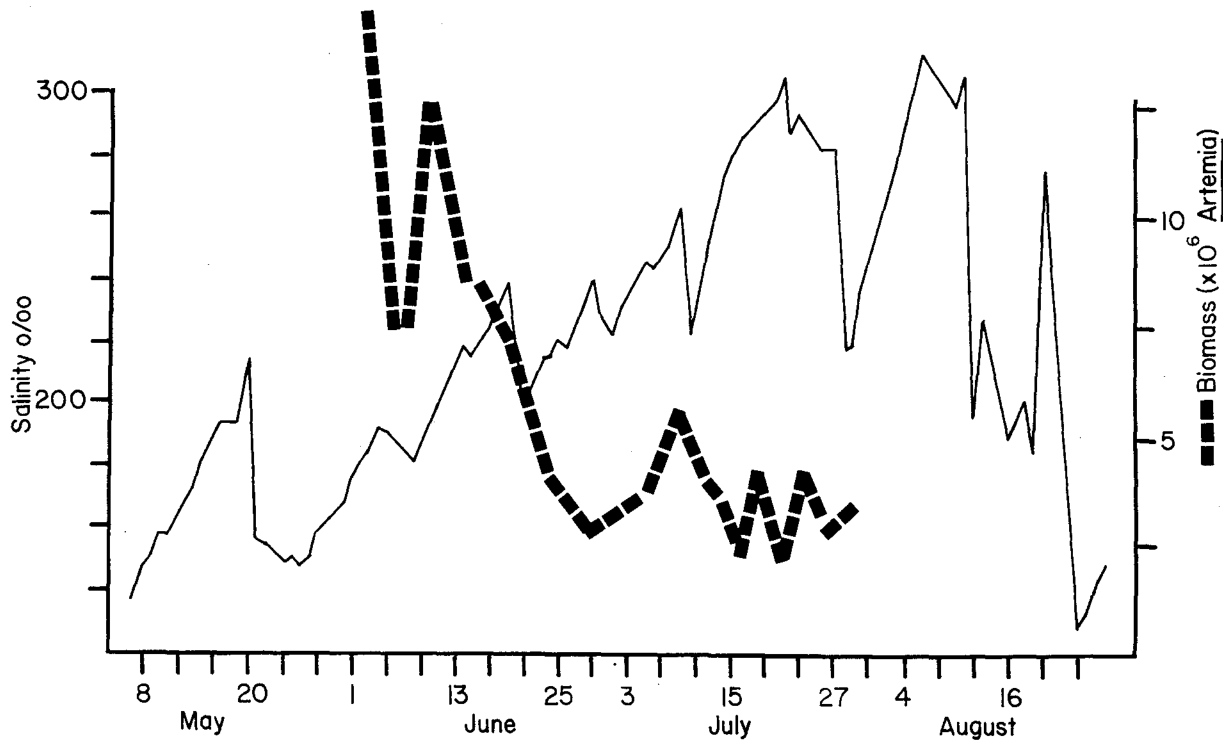


Figure 16 -- Mean Daily Salinity and Reproductive Mode Data for Experimental Pond 1



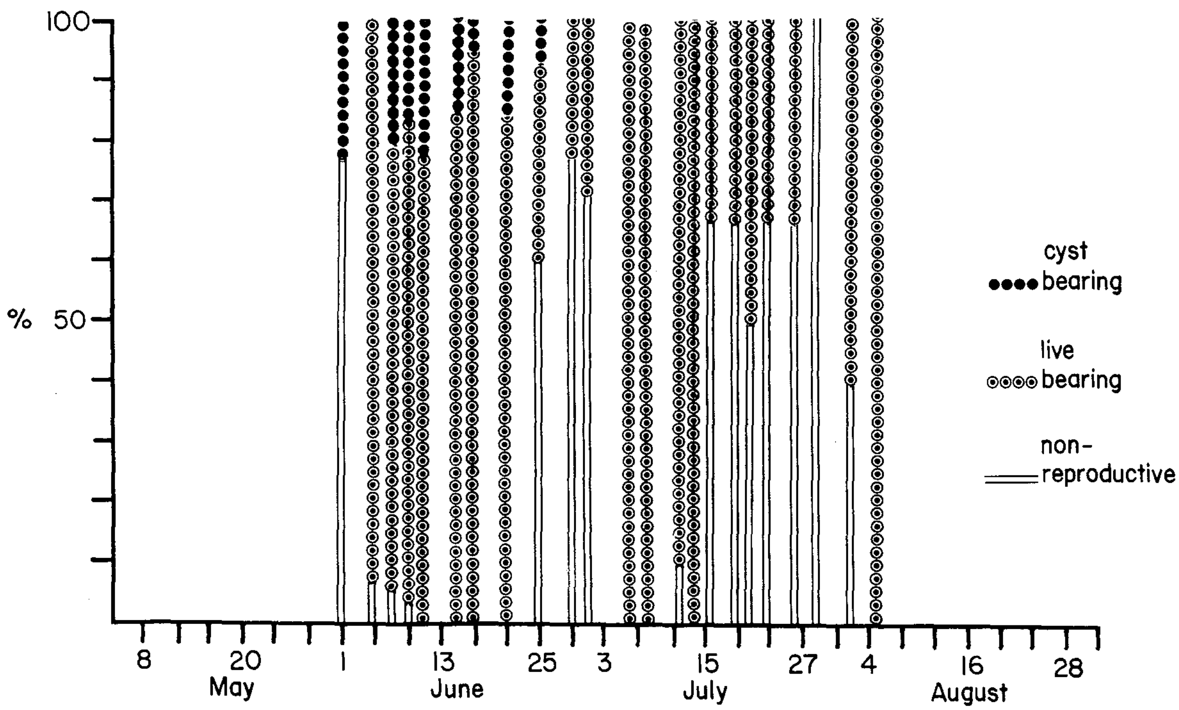
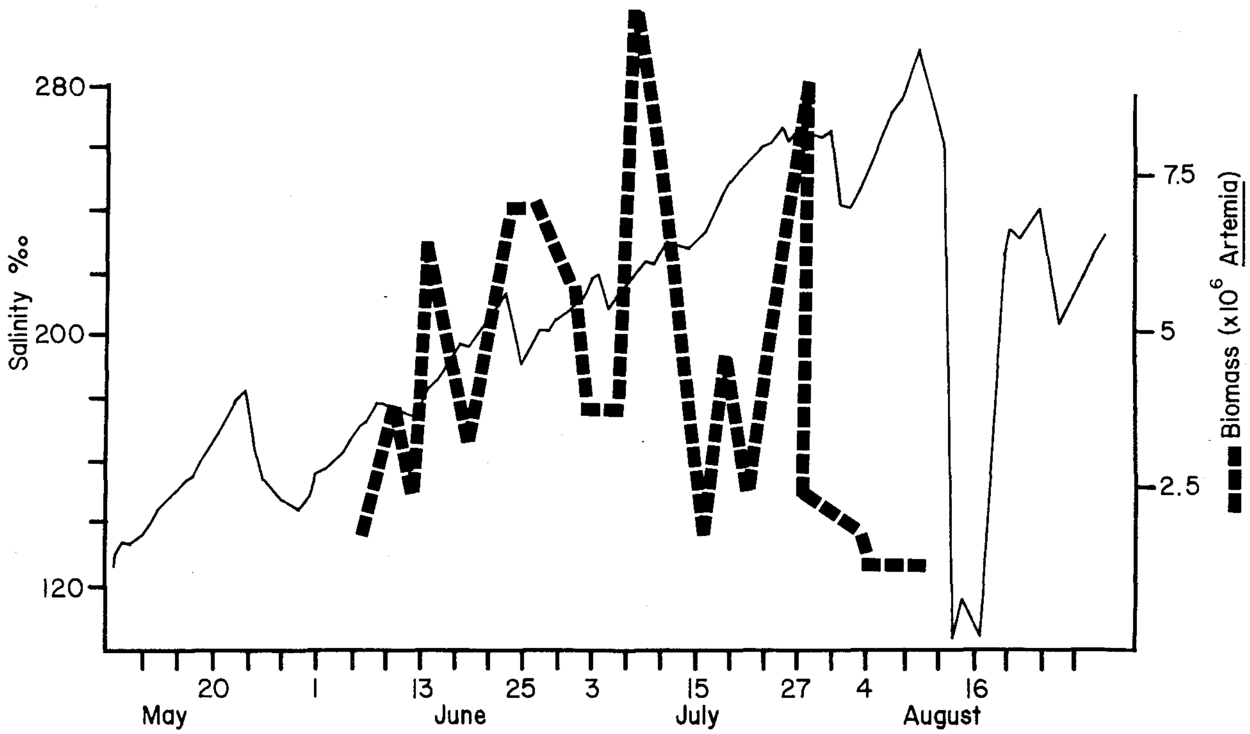


Figure 17 -- Mean Daily Salinity and Reproductive Mode Data for Experimental Pond 2



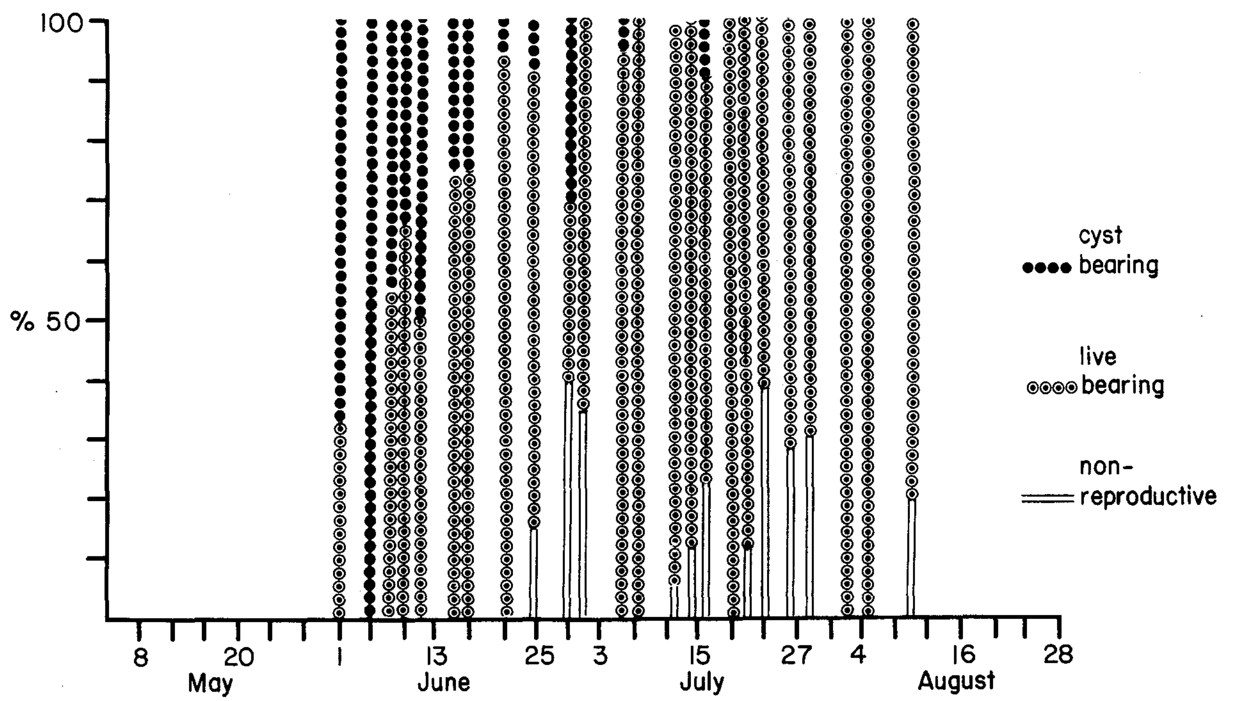
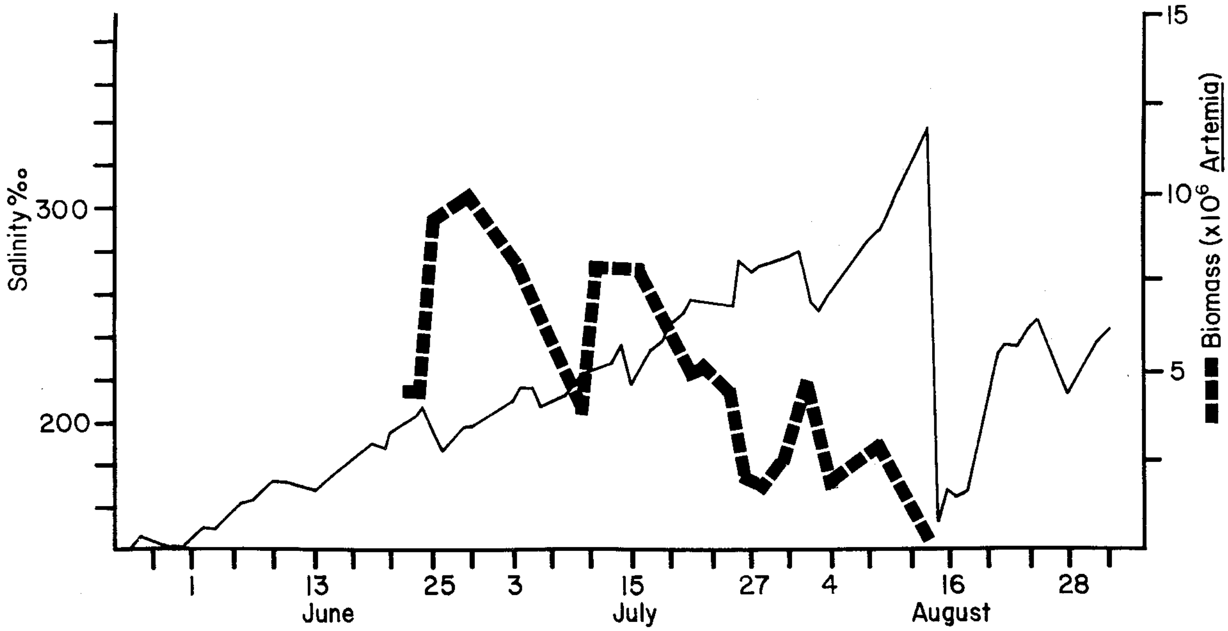


Figure 18 -- Mean Daily Salinity and Reproductive Mode Data for Experimental Pond 3



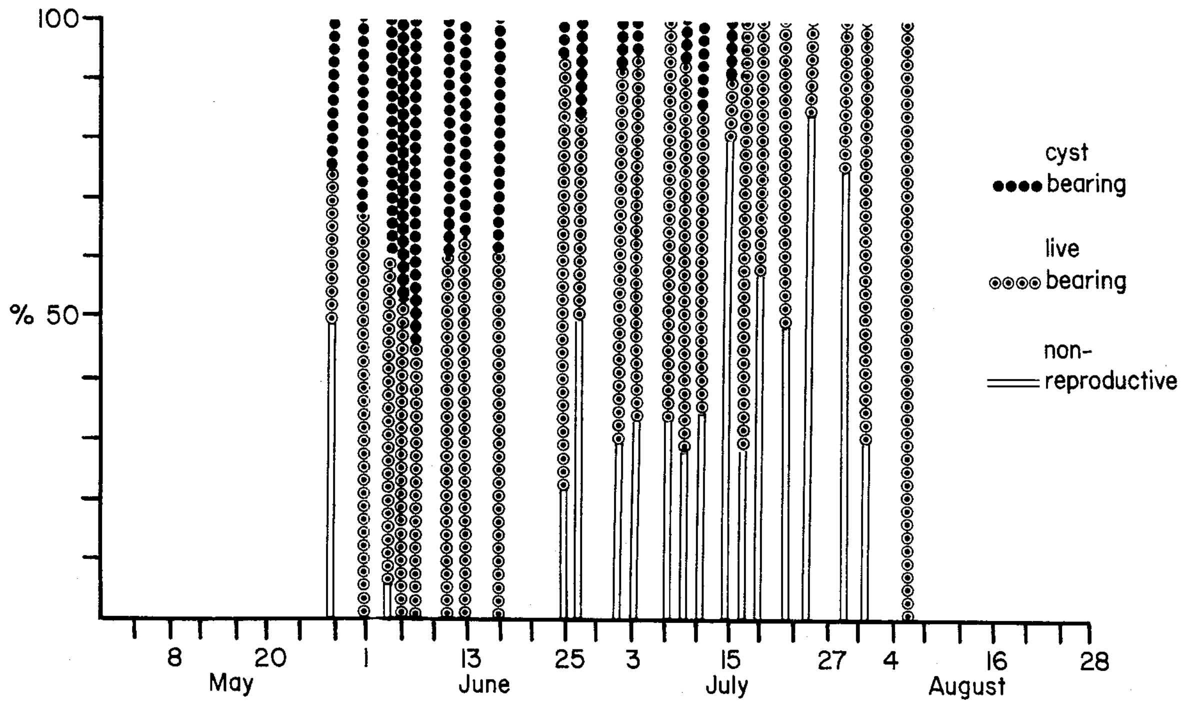
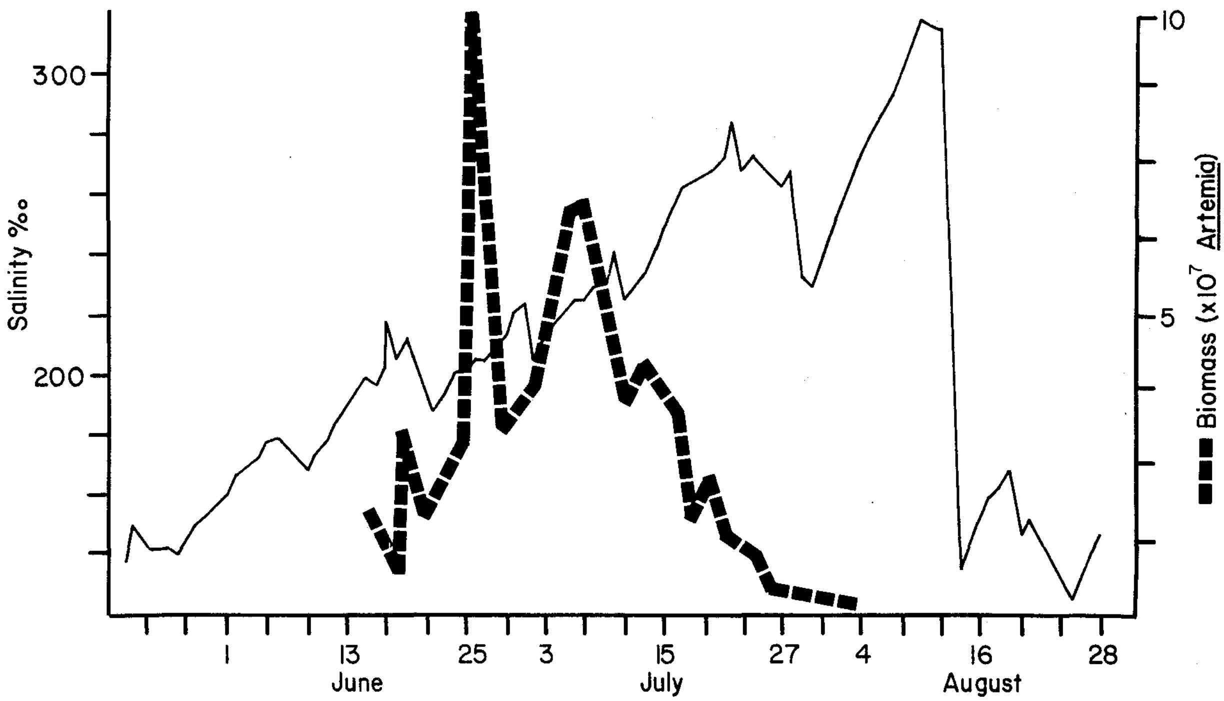


Figure 19 -- Mean Daily Salinity and Reproductive Mode Data for Experimental Pond 4



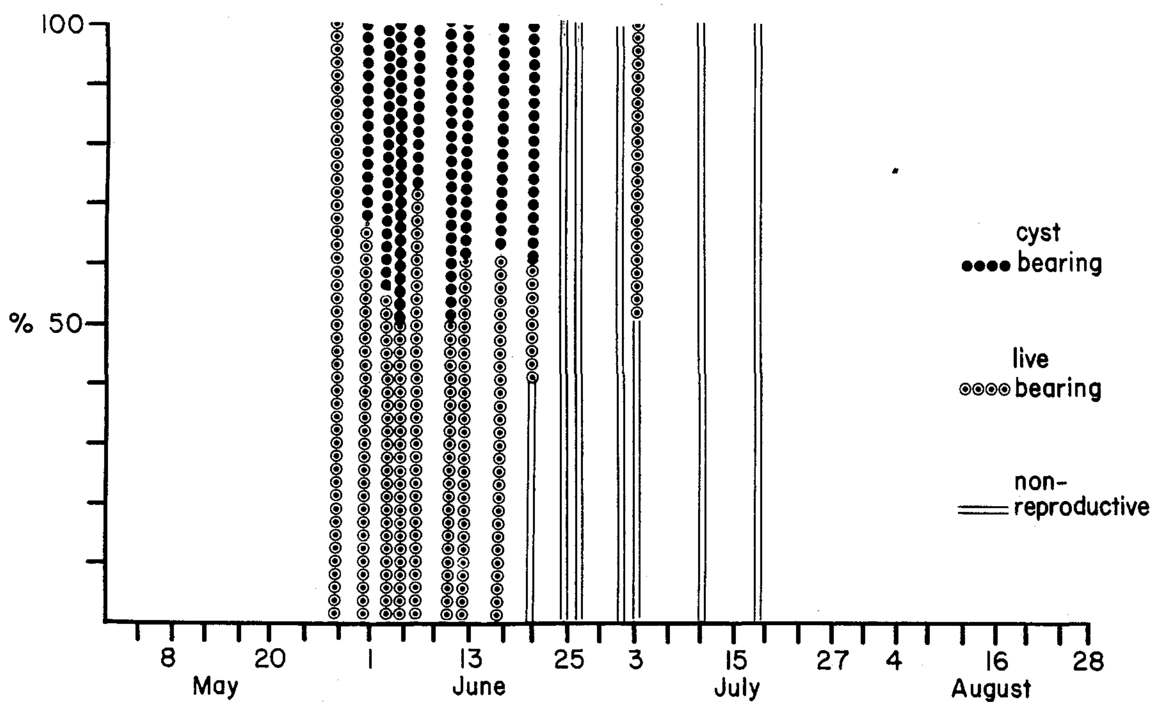


Figure 20 -- Mean Daily Salinity and Reproductive Mode Data for Experimental Pond 5

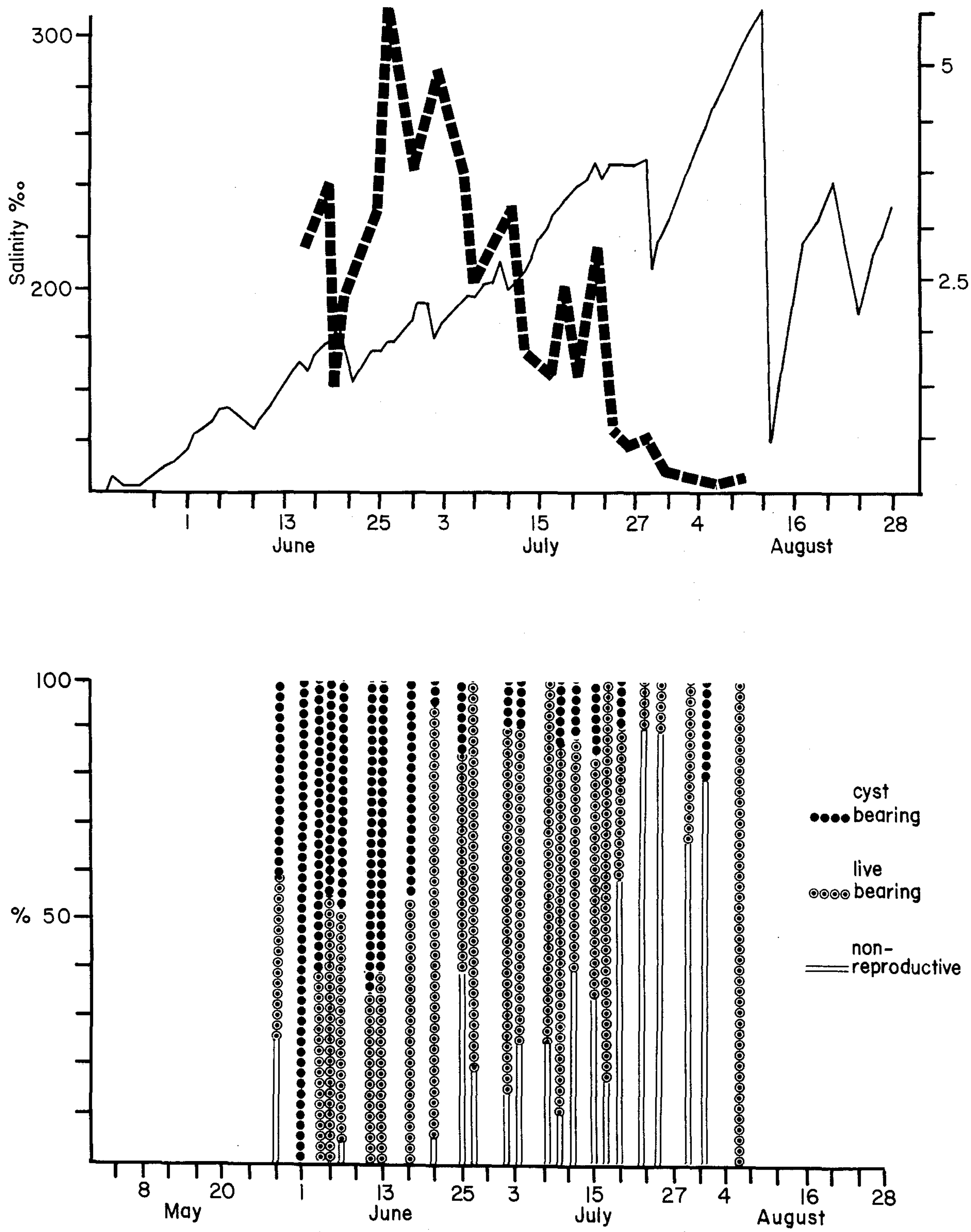
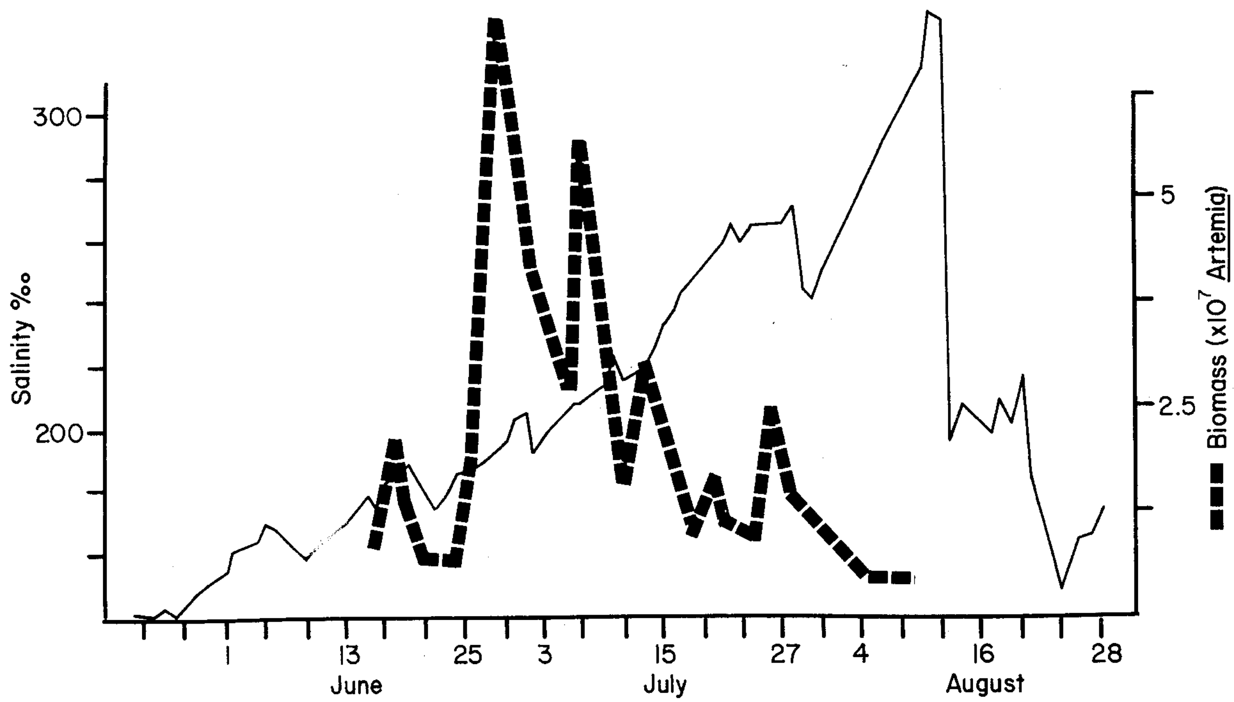


Figure 21 -- Mean Daily Salinity and Reproductive Mode Data for Experimental Pond 6



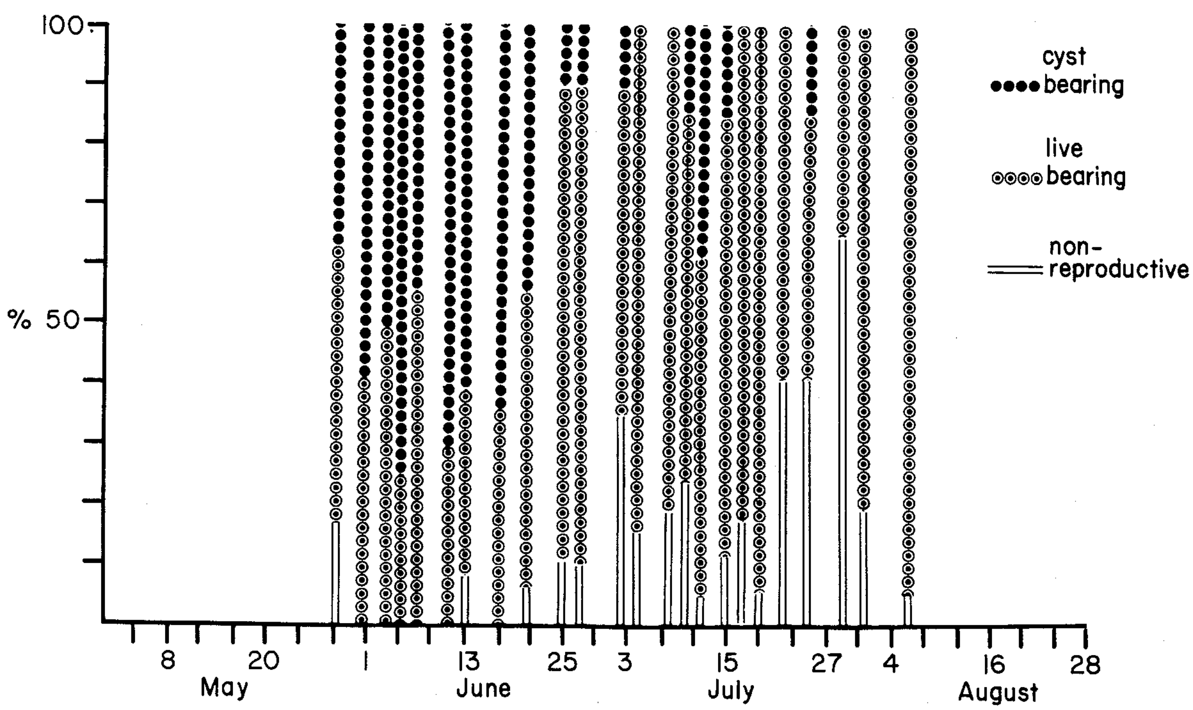


Table 4: Results of Dissolved Oxygen Measurements in Experimental Ponds

Pond Number	Location	Date	Time	D.O.(ppm)
1	bottom	6/6/84	1000	3.7
1	bottom	6/6/84	1000	4.4
1	bottom	6/6/84	1000	4.2
1	bottom	6/6/84	1000	3.0
1	surface	6/6/84	1000	3.4
1	surface	6/6/84	1000	4.5
1	surface	6/6/84	1000	3.1
1	mid-water	7/6/84	1100	1.7
1	mid-water	7/6/84	1100	1.4
1	mid-water	7/6/84	1100	2.6
1	mid-water	7/6/84	1315	2.0
1	mid-water	7/6/84	1315	2.9
1	mid-water	7/6/84	1315	2.2
1	mid-water	8/6/84	0900	3.2
1	mid-water	8/6/84	0900	2.7
1	mid-water	8/6/84	0900	3.3
1	mid-water	8/6/84	1300	2.8
1	mid-water	8/6/84	1300	2.6
1	mid-water	8/6/84	1300	2.2
1	mid-water	25/6/84	1030	1.4
1	mid-water	25/6/84	1030	1.2
1	mid-water	18/7/84	1200	0.7
1	mid-water	18/7/84	1200	1.0
1	bottom	24/7/84	0600	1.3
1	surface	24/7/84	0600	0.9
1	surface	24/7/84	0600	1.0
1	bottom	24/7/84	0600	1.0
1	bottom	24/7/84	0600	1.2
1	surface	24/7/84	0600	1.2
1	surface	24/7/84	0600	1.2
1	surface	24/7/84	1600	1.6
1	surface	24/7/84	1600	1.0
1	bottom	24/7/84	1600	0.9
1	bottom	24/7/84	1600	1.1
1	bottom	21/8/84	1400	0
1	bottom	21/8/84	1400	1.9
2	mid-water	25/6/84	1030	1.7
2	mid-water	25/6/84	1030	2.2
2	mid-water	18/7/84	1200	0.6
2	mid-water	18/7/84	1200	0.8
2	mid-water	31/7/84	0600	0.7
2	surface	31/7/84	0600	0.6
2	surface	31/7/84	0600	0.6
2	bottom	31/7/84	0600	0.6
2	bottom	31/7/84	0600	0.6

Table 4 (continued)

Pond Number	Location	Date	Time	D.O.(ppm)
2	bottom	21/8/84	1400	1.3
2	bottom	21/8/84	1400	0
3	mid-water	25/6/84	1030	1.4
3	mid-water	25/6/84	1030	1.9
3	mid-water	16/7/84	1600	1.4
3	mid-water	16/7/84	1600	1.5
3	bottom	21/8/84	1400	0.5
3	bottom	21/8/84	1400	0
4	mid-water	26/6/84	1030	1.6
4	mid-water	26/6/84	1030	0.9
4	mid-water	16/7/84	1600	1.5
4	mid-water	16/7/84	1600	1.4
4	bottom	21/8/84	1400	0
4	bottom	21/8/84	1400	0
5	mid-water	7/6/84	1100	1.8
5	mid-water	7/6/84	1100	1.0
5	mid-water	7/6/84	1315	2.1
5	mid-water	7/6/84	1315	2.1
5	mid-water	8/6/84	0900	2.8
5	mid-water	8/6/84	0900	2.5
5	mid-water	8/6/84	1300	1.8
5	mid-water	8/6/84	1300	2.8
5	mid-water	8/6/84	1300	0.8
5	mid-water	8/6/84	1300	2.5
5	mid-water	9/6/84	1330	1.8
5	mid-water	9/6/84	1330	1.6
5	mid-water	9/6/84	1330	2.5
5	mid-water	9/6/84	1330	2.2
5	mid-water	9/6/84	1330	2.5
5	mid-water	9/6/84	1330	2.4
5	mid-water	26/6/84	1030	0.7
5	mid-water	26/6/84	1030	1.2
5	mid-water	16/7/84	1600	0.8
5	mid-water	16/7/84	1600	0.9
5	bottom	21/8/84	1400	0.8
5	bottom	21/8/84	1400	0.2
6	mid-water	26/6/84	1030	1.4
6	mid-water	26/6/84	1030	0.8
6	mid-water	16/7/84	1600	0.7
6	mid-water	16/7/84	1600	0.7
6	bottom	21/8/84	1400	0
6	bottom	21/8/84	1400	0

Table 5: Results of pH Determinations in Experimental Ponds

Pond Number	Location	Date	Time	pH
1	midwater	25/6/84	1200	7.8
1	bottom	10/7/84	1030	7.6
1	surface	11/7/84	1330	7.6
1	surface	12/7/84	1310	7.6
1	surface	13/7/84	1530	7.6
1	bottom	17/7/84	1130	7.5
2	midwater	25/6/84	1200	7.8
2	surface	10/7/84	1030	7.6
2	surface	11/7/84	1330	7.6
2	surface	12/7/84	1310	7.6
2	surface	13/7/84	1530	7.6
2	bottom	17/7/84	1130	7.6
3	midwater	25/6/84	1200	7.8
3	surface	10/7/84	1030	7.6
3	surface	11/7/84	1330	7.6
3	surface	12/7/84	1310	7.7
3	surface	13/7/84	1530	7.6
3	bottom	17/7/84	1130	7.6
4	bottom	10/7/84	1030	7.6
4	surface	11/7/84	1330	7.6
4	surface	12/7/84	1310	7.6
4	surface	13/7/84	1530	7.6
4	bottom	17/7/84	1130	7.6
5	surface	10/7/84	1030	7.5
5	surface	11/7/84	1330	7.6
5	surface	12/7/84	1310	7.6
5	surface	13/7/84	1530	7.6
5	bottom	17/7/84	1130	7.5
6	surface	10/7/84	1030	7.4
6	surface	11/7/84	1330	7.5
6	surface	12/7/84	1310	7.5
6	surface	13/7/84	1530	7.5
6	bottom	17/7/84	1130	7.4

Table 6: Results of Biochemical Oxygen Demand Determinations in Experimental Ponds

Pond No.	Date	Initial DO (ppm)	Final DO (ppm) Light Bottle	Final DO (ppm) Dark Bottle	BOD (1)
1	1/8/84	1.8	1.0	0	1.8
1	1/8/84	1.5	1.1	0	1.5
2	1/8/84	1.1	0.8	0.4	0.7
2	1/8/84	1.0	0.8	0.4	0.6
3	2/8/84	0.8	0.5	0.3	0.5
3	2/8/84	1.0	0.4	0.2	0.8
4	2/8/84	1.8	0.3	0.2	1.6
4	2/8/84	1.5	0.4	0.2	1.3
5	4/8/84	0.7	1.0	0.9	-
5	4/8/84	0.8	1.4	0.9	-
6	4/8/84	0.8	0.8	0.3	0.5
6	4/8/84	0.7	0.7	0.3	0.4

(1) -- ppm per day; in cases where no dissolved oxygen was detected in dark bottles these figures are minima

Table 7: Results of Duplicate Analyses for Iron and Nutrient Content of Experimental Ponds Before and After Manipulation (ug-at/l)

Pond	Nitrate + Nitrite Nitrogen	Nitrite Nitrogen	Ammonia Nitrogen	Phosphate	Fe
Pre-Manipulation					
1	1.0	*	258.7	5.1	+
	0.8		260.2		+
2	0.4	*	18.6	*	+
	0.4		17.7		+
3	0.3	*	39.3	*	*
	0.3		39.7		*
4	2.5	*	207.5	0.4	*
	2.5		212.5		*
5	0.3	*	32.0	*	*
	0.3		32.0		*
6	0.4	*	19.1	*	*
	0.4		19.1		*
Post-Manipulation					
1	0.2	*	5.5	*	*
	0.1		5.5		*
2	0.3	0.1	3.3	*	*
	0.3		2.3		*
3	0.2	*	2.1	*	*
	0.1		2.1		*
4	0.3	*	10.2	*	*
	0.2		10.5		*
5	0.2	0.1	*	*	*
	0.1	0.1	*		*
6	0.3	*	4.3	*	*
	0.2		4.2		*

* -- undetectable
+ -- trace at limit of detection

Population size declined during the study period, and appeared roughly correlated with rising salinity. It should be noted that *Artemia* populations in the main pond and other ponds used by the shrimp farm did not exhibit the same decline, and that the salinity in the latter ponds did not rise to the levels attained in the experimental enclosures. Nutritional depletion in the enclosed areas thus seems a less likely cause for the decline in population size than rising salinity.

Survival and growth of penaeid shrimp postlarvae fed on two strains of *Artemia* are summarized in Table 8. *Artemia* from San Francisco Bay provided consistently better growth and survival than St. Kitts cysts, but these differences are not statistically significant.

Cyst hatching percentage data are presented in Table 9. St. Kitts cysts are at least equal to the commercial product tested, even though processing and storage procedures were probably not optimal.

Qualitative descriptions provided by the algologist consultant for algae in various samples are presented in Table 10.

D. Investigations at the University of the West Indies

Survival data on Reference and St. Kitts *Artemia* reared on various diets are summarized in Table 11. No diet was definitely superior for St. Kitts brine shrimp but Reference *Artemia* clearly did better on the algal diet.

Data on hatching percentage of St. Kitts and Reference *Artemia* cysts at various salinities are presented in Table 12. St. Kitts cysts demonstrated a much lower percentage hatch than Reference material, probably because the former were not processed to remove non-viable cysts during the collection process. Though samples sizes are too small to allow statistical analysis, there are indications that 60 o/oo may be close to optimum hatching salinity for both St. Kitts and Reference cysts.

Data from multivariate experiments are presented in Table 13. Table 14 summarizes analyses of variance of these data. High within-group variability was observed in both reference and St. Kitts *Artemia*. Reduced oxygen levels could not be reliably maintained in the cultures with available equipment and facilities. Low hatch frequencies and high mortality prevented the anticipated number of replicates in some cases, and the full multivariate analysis originally contemplated was not

Table 8: Survival and growth of *Penaeus vannamei* postlarvae reared on two strains of *Artemia*

Source of *Artemia*	Mean Size at End of Experiment (mm)	Standard Deviation	Survival (%)
St. Kitts	10.4	1.47	41.9
St. Kitts	10.2	2.08	19.4
St. Kitts	12.4	1.98	12.9
San Francisco Bay	11.1	2.50	54.8
San Francisco Bay	12.1	1.54	41.9
San Francisco Bay	13.0	1.59	35.5

Table 9: 36-hour Hatching Frequency of *Artemia* from Various Sources

Source	Mean % Hatch (5 replicates)	Standard Deviation
Great Salt Pond (St. Kitts)	72.4	12.4
Experimental Pond 5 29 May, 1984	27.0	6.2
Experimental Pond 5 21 July, 1984	54.0	5.9
San Francisco Bay	15.8	3.56

Table 10: Characterization of Algae Samples

Pond (1)	Date (2)	Chlorophyta mf	sf	lf	src	Cyanophyta bgu	O	S	L	cu	D	U
P1	8/8/84			XXXX			X					
P1	30/8/84		XXXX			XXX	XXX					
P1-B	8/8/84				X		XXXX	X				
P1-B	30/8/84				X	XXX	XXX			XXXX		
P2	8/8/84			X		XXX	XXX			XXX		
P2	30/8/84					XXXX		XX				
P2-B	8/8/84			X		XXX	XXX			XXX		
P2-B	30/8/84				XX	XX	XXXX	XXXX		XX		
P3	8/8/84		X			XXX	XXX	XXX				
P3	30/8/84		XXXX			XXX	XX					
P3-B	8/8/84			X		XXX	XXXX	XX		XX		
P3-B	30/8/84				X	XX	XXXX			XXXX		
P4	8/8/84			XXX		XXX	XXX					
						X		X				
P4-B	8/8/84					XXXX	XXXX			XXX		
							XXXX					
P5	8/8/84			XXXX								
P5	30/8/84		XXXX			XXX	XXX					
P5-B	8/8/84			XX		XX	XXX			XXXX		
P5-B	30/8/84					XXX	XXXX					
P6	8/8/84			XX			XXX					
P6	30/8/84		XXXX									X
P6-B	8/8/84					XXX	XXX	XXX		XXXX		
P6-B	30/8/84						XXXX	XXX		XXX		

TABLE 10 (continued)

Pond (1)	Date (2)	Chlorophyta mf	sf	lf	src	Cyanophyta bgu	O	S	L	cu	D	U
AN4	8/5/84			XXX			XXXX				X	
AX1	8/2/84			X		X	X			X		
AX1	8/5/84			X			XXXX	X			X	
AX1	28/7/84						XXX	XX		XX		
AX1	12/8/84										X	
AX2	7/7/84									XXXX		
AX2	4/5/84											X
AX2	8/5/84						X					
AX2	28/7/84										X	
AX2	11/8/84										X	X
BV12	20/6/84			X			XXXX				X	
BV12	13/8/84			XXXX			XX					

mf = microflagellate, less than 5 u length, probably Chlorophyta
sf = small flagellate, 5 - 10 u length, probably Chlorophyta
lf = large flagellate, more than 10 u length, probably Chlorophyta
src = spherical thick-walled resting cell, probably Chlorophyta
bgu = blue-green unicells (Cyanophyta)
O = filamentous, uniseriate; Oscillatoria sp. (Cyanophyta)
S = spiral filamentous; Spirulina sp. (Cyanophyta)
L = filamentous sheathed alga; Lyngbya sp. (Cyanophyta)
cu = clumped unicells forming sub-spherical colonies to 200 u diameter; common in sediment and have been seen to pass through the gut of Artemia apparently undigested; possibly Cyanophyta
D = diatoms (Bacillariophyta); benthic forms
U = unidentified unicells; non-motile
X = present but rare; XX = present, not common; XXX = common; XXXX = dominant

Table 11: Survival and Time to Maturity of St. Kitts and Reference *Artemia* Reared on Various Diets

35 o/oo					50 o/oo				
Food (1)	Number of *Artemia* D-1	D-14	D-21	Days to Maturity	Food (1)	Number of *Artemia* D-1	D-14	D-21	Days to Maturity
Reference *Artemia*									
WG	120	1	0	15	WG	29600	0	0	-
WG	120	21	20	14	WG	250	0	0	-
WG	120	9	8	14	WG	215	0	0	-
PL	120	94	74	9	PL	29600	78	51	9
PL	120	59	51	9	ST	29600	0	0	-
PL	120	85	81	9	ST	250	0	0	-
					ST	200	0	0	-
					NA	29600	6	0	-
					NA	250	1	0	-
St. Kitts *Artemia*									
WG	90	4	4	14	WG	70500	21	7	13
WG	90	12	5	10	PL	70500	29	19	10
WG	90	11	7	14	ST	70500	0	0	-
ST	90	10	8	14	ST	250	9	4	-
ST	90	11	5	10	NA	70500	0	0	-
ST	90	3	3	14	NA	250	0	0	-
PL	74	1	1	12	NA	160	0	0	-
PL	74	7	7	7					
PL	74	12	9	12					

(1) -- WG = Wheat germ; PL = *Tetraselmis* sp.; ST = *Nannochloris stomus*; NA = *Nannochloropsis salina*

Table 12: Hatching Frequency of St. Kitts and Reference *Artemia* Cysts at Various Salinities

	St. Kitts Artemia			Reference Artemia		
Salinity o/oo	Number of Cysts Initial	Number of Cysts Final	Hatch %	Number of Cysts Initial	Number of Cysts Final	Hatch %
35	31,000	2,000	6.5	75,000	56,500	75.3
35	45,500	3,500	7.7	78,000	67,000	85.9
35	70,500	1,000	1.4	74,000	42,000	56.8
35	21,500	0	0			
50	33,300	1,300	3.9			
60	58,000	2,930	5.1	152,000	110,300	72.6
90	58,000	0	0	152,000	8,730	5.7
120	58,000	67	0.1	152,000	0	0

Table 13: Effect of Salinity and Temperature Variation on Time to Maturity, Time to Reproduction, and Survival of Reference and St. Kitts *Artemia*

Salinity o/oo	Temperature C	Days to Maturity	Days to Reproduction	Survival %
Reference *Artemia*				
60	25	12	41	5
60	25	10	25	6.75
60	25	19	45	0.625
60	25	17	37	4.625
90	25	17	37	7.125
90	25	20	39	4.25
90	25	20	27	5.125
120	25	--	--	0
120	25	--	--	0
120	25	20	34	2.6
120	25	20	27	1.4
60	30	16	17	1
60	30	14	17	0.125
60	30	--	--	0
60	30	14	NA	0.25
60	30	9	29	21.84
60	30	10	29	23.3
60	30	10	31	4.35
60	30	10	31	3.42
90	30	11	17	1.5
90	30	8	17	0.875
90	30	14	NA	0.125
90	30	8	17	0.75
90	30	NA	30	10
90	30	NA	NA	5.8
90	30	9	28	3.2
90	30	10	33	4
120	30	--	--	0
120	30	--	--	0
120	30	--	--	0
120	30	--	--	0
120	30	--	--	0
120	30	NA	NA	0.125
120	30	NA	32	4.35
120	30	NA	29	8.37
120	30	NA	30	4
120	30	NA	29	3.46

Table 13 (continued)

Salinity o/oo	Temperature C	Days to Maturity	Days to Reproduction	Survival %
Reference *Artemia*				
60	35	--	--	0
60	35	--	--	0
60	35	--	--	0
60	35	--	--	0
90	35	--	--	0
90	35	--	--	0
90	35	--	--	0
90	35	--	--	0
120	35	--	--	0
120	35	--	--	0
120	35	--	--	0
120	35	--	--	0
St. Kitts *Artemia*				
60	25	12	21	8
60	25	14	26	6.875
60	25	19	25	6.25
60	25	14	19	4.625
60	25	9	28	74.5
60	25	18	30	59
90	25	26	30	5.75
90	25	19	25	6.25
90	25	19	21	6.375
90	25	18	28	60
90	25	18	28	61
90	25	9	28	81
120	25	21	25	6.125
120	25	21	25	5.25
120	25	21	25	8.25
120	25	21	25	7.5
120	25	9	18	0.5
120	25	9	15	56

Table 13 (continued)

Salinity o/oo	Temperature C	Days to Maturity	Days to Reproduction	Survival %
60	30	10	24	6.125
60	30	11	13	6
60	30	10	24	3.125
60	30	10	24	4.875
60	30	7	NA	0.48
60	30	8	23	1.9
60	30	8	23	1.47
60	30	9	23	0
60	30	9	23	0.93
90	30	--	--	0
90	30	--	--	0
90	30	--	--	0
90	30	10	NA	0.5
90	30	10	NA	1.5
60	35	13	30	0
60	35	12	18	1.25
60	35	14	29	1.375
60	35	14	29	1.125
60	35	9	16	1.5
60	35	--	--	0
60	35	--	--	0
60	35	15	NA	2
90	35	10	21	5
90	35	10	22	2
90	35	10	22	12
90	35	10	22	5
120	35	10	NA	0.5
120	35	--	--	0
120	35	--	--	0
120	35	--	--	0

Table 14: Summary of Analyses of Variance of Survival Among Groups of *Artemia* at Various Salinities and Temperatures

Comparison	Source of Variation						F
	Among Groups			Within Groups			
	df	SS	MS	df	SS	MS	
Reference *Artemia*, 30 C -- 60:90:120 o/oo	2	154.45	77.22	21	1429.7	68.08	1.13
St. Kitts *Artemia*, 25 C -- 60:90:120 o/oo	2	783.23	391.62	15	6028.0	401.87	0.974
St. Kitts *Artemia*, 35 C -- 60:90 o/oo	1	173.24	173.24	10	189.6	18.99	9.14*
St. Kitts *Artemia*, 60 o/oo -- 25:30:35 C	2	2136.89	1068.44	20	2501.1	125.06	8.54*
St. Kitts *Artemia*, 90 o/oo -- 25:35 C	1	1217.32	1217.32	8	2746.8	343.25	3.55

possible. Significant differences were observed in animals reared at different temperatures under low salinity conditions, with lowest temperature (25 C) coinciding with highest survival rates. When salinity was varied at a constant temperature of 25 C, no significant differences in survival were detected. At 35 C survival was better at 90 o/oo than at 60 o/oo but total mortality resulted at 120 o/oo. Pending further study, it is suggested that optimum temperature for St. Kitts *Artemia* is between 25 and 30 C, and optimum salinity is in the vicinity of 90 o/oo.

Results of attempts to increase cyst production are summarized in Table 15. No statistically significant differences were observed in cyst production between the two groups over the total experimental period, nor between the two groups during the first week of this period. Both groups showed significantly higher ($P < 0.005$; $P < 0.001$) cyst production during the second half of the experiment. The group exposed to reduced salinity, however, had significantly higher ($P < 0.025$) cyst production than the control group, supporting the hypothesis that sudden salinity reduction results in increased cyst production.

Reproductive capacity of ten adult female *Artemia* from St. Kitts is summarized in Table 16. Because these figures were obtained from animals reared at a single salinity, it cannot be determined whether they represent maximum reproductive capacity.

E. Biochemical Studies

Fatty acid analyses are summarized in Table 17. Early samples contained almost no long chain polyunsaturated fatty acids, with the exception of samples collected in the British Virgin Islands. Later samples (which were partially extracted in the field) showed improved PUFA profiles in all sites. In addition, algae cultured from hypersaline ponds also were found to have concentrations of PUFA which would be nutritionally useful in mariculture operations for a variety of species.

Tracings of densitometric scans of gels produced by isoelectric focussing electrophoresis of selected *Artemia* samples are presented in Figure 23. All Caribbean samples were significantly different from Reference *Artemia* for the enzymes tested. Moreover, *Artemia* from experimental pond 5 were slightly different from *Artemia* collected a year earlier in St. Kitts. The latter observation probably indicates isozyme adaptations within the population, but the differences between Caribbean and Reference *Artemia* are more likely indicative of different strains. The Caribbean

Table 15: Effect of Sudden Salinity Reduction on Reproductive Mode in St. Kitts *Artemia*

Week 1			Week 2		
Salinity o/oo	Cysts Produced	Larvae Produced	Salinity o/oo	Cysts Produced	Larvae Produced
Group I					
120-150	7	0	60-90	43	0
120-150	4	0	60-90	23	0
120-150	7	0	60-90	19	0
120-150	14	0	60-90	34	0
120-150	13	0	60-90	31	0
120-150	14	0	60-90	19	0
120-150	11	0	60-90	24	0
120-150	6	0	60-90	34	0
120-150	1	1	60-90	14	27
120-150	0	8	60-90	33	0
Mean	7.7	0.9		27.4	2.7
S.D.	5.2	2.5		9.0	8.5
Group II					
130-150	0	0	150	25	0
130-150	10	0	150	32	0
130-150	6	0	150	14	0
130-150	0	0	150	0	4
130-150	1	0	150	17	0
130-150	2	0	150	14	18
130-150	4	0	150	17	0
130-150	2	0	150	23	0
130-150	8	0	150	10	6
130-150	9	0			
Mean	4.2	0		16.9	3.1
S.D.	3.8	0		9.2	6.0

Analyses of Variance --

Group I Week 1:Group I Week 2

Between Groups:	df = 1	SS = 1940.45	MS = 1940.45
Within Groups:	df = 18	SS = 966.5	MS = 53.69
			F = 36.14*

Group II Week 1:Group II Week 2

Between Groups:	df = 1	SS = 616.05	MS = 616.05
Within Groups:	df = 18	SS = 1075.5	MS = 59.75
			F = 10.31*

Group I Week 1:Group II Week 1

Between Groups:	df = 1	SS = 64.8	MS = 64.8
Within Groups:	df = 18	SS = 329	MS = 18.27
			F = 3.55

Group I Week 2:Group II Week 2

Between Groups:	df = 1	SS = 744.2	MS = 744.2
Within Groups:	df = 18	SS = 1664	MS = 92.44

Table 16: Reproductive Capacity of St. Kitts *Artemia* Reared on *Tetraselmis* sp. at 60 o/oo and 30 C

Observation Period (days)	Cysts Produced	Nauplii Produced	Total
21	39	55	94
34	93	20	113
34	21	14	35
19	24	84	108
34	116	9	125
34	81	37	118
34	54	21	75
34	28	22	50
18	9	64	73
34	49	38	87
Mean	51.4	36.4	87.8
S.D.	34.9	24.4	29.8

Table 17: Summary of Fatty Acid Analyses (percentage composition)

Source:	AN4	AN4	AN4	AX1	AX1	AX1
Date Collected:	8/12/83	6/6/84	9/7/84	17/12/83	17/12/83	19/12/83
Specimen Type:	A	A	A	A	A	A
Fatty Acid						
C10:0			0.09			
C12:0	1.97		0.03	0.87	1.80	0.80
C14:0	0.26	1.63	1.22	5.85	8.59	4.78
C14:1w9		5.1	4.66			
C14:2	16.83			1.35	1.03	0.87
C15:1		1.94				
C16:0		18.13	17.35	23.53	21.06	21.01
C16:1		10.47	14.3	17.16	18.84	15.40
C16:2	6.39	1.88	2.30			2.65
C17:0		2.50				
C18:0	42.81	5.77		4.56	5.94	4.38
C18:1w9	16.97	31.13	33.39	31.26	35.60	39.54
C18:2w6	0.05	6.12	8.77	12.57	5.05	9.96
C18:3w3		5.32	1.09	0.16		
C20:1w9	1.18	0.47	6.19			
C18:4			0.71			
C20:2			0.11			
C21:0		0.93				
C22:1w9				0.38	0.97	0.62
C24:1w9	0.04					
C20:3		2.81	0.04			
C20:4			2.81			
C20:5w3		2.16	2.82		0.79	
C22:4			0.84			
C22:5w3		2.74				
C22:6w3			2.93			

Table 17 (continued)

Source:	AX1	AX1	AX2	AX2	AX4	AX8
Date Collected:	29/7/84	8/5/84	17/12/83	7/7/84	17/12/83	18/12/83
Specimen Type:	A	A	C	A	A	A
Fatty Acid						
C10:0				0.16		
C12:0			1.06		1.39	3.23
C14:0		1.63	2.53	1.24	1.94	
C14:1w9		7.23		7.25		
C14:2					0.21	0.16
C15:0	12.34					
C15:1		3.24				
C16:0		17.38	19.86	12.71	18.03	18.89
C16:1	18.53	12.49	17.63	12.63	17.49	22.31
C16:2	4.14	4.52	9.32	5.18		
C17:0	11.74	4.98		5.74		
C18:0	3.85	4.38		5.60	5.51	6.70
C18:1w9		27.35	40.06	24.56	43.70	39.02
C18:2w6	27.47	10.17	7.93	4.61	3.53	3.28
C18:2w9				8.44		
C18:3w3	1.48	0.81		4.16	0.18	0.47
C18:3w6	9.09					
C20:1w9						
C18:4						
C20:2	0.74			0.28		
C21:0	0.68					
C22:1w9			1.03		5.14	4.08
C24:1w9						
C20:3	1.20	0.68		0.07		
C20:4	1.74					
C20:5w3	2.12	1.86	0.59	0.89	2.88	1.87
C22:4	0.77					
C22:5w3	0.15					
C22:6w3	1.96	2.90		2.59		

Table 17 (continued)

Source:	BV12	BV12	BV13	BV13	BV13	BV13	BV13
Date Collected:	13/11/83	13/11/83	14/11/83	20/2/84	20/2/84	29/2/84	7/3/84
Specimen Type:	A	A	A	A	A	A	A
Fatty Acid							
C10:0							
C12:0	8.55		1.78				
C14:0		6.23		3.26	8.97	2.10	1.75
C14:1w9	0.98	0.98	0.28				
C14:2							
C15:1							
C16:0	27.20	27.25	17.65	20.42	31.59	18.02	15.80
C16:1	26.17	19.38	17.93	20.24	19.20	18.57	16.11
C16:2							
C17:0							
C18:0	8.43	11.59	9.35	4.59	2.80	4.76	6.94
C18:1w9	13.71	26.83	37.61	47.17	35.49	52.52	52.35
C18:2w6	1.92	1.47	4.94	3.27	1.95	3.42	4.68
C18:3w3	0.57	0.28	0.30				
C20:1w9		0.18		1.04		0.61	1.60
C18:4							
C20:2							
C21:0							
C22:1w9	4.82	2.51	5.84				
C24:1w9							
C20:3							
C20:4							
C20:5w3	7.03	3.28	4.15				0.77
C22:4							
C22:5w3							
C22:6w3							

Table 17 (continued)

Source:	BV13	BV14	BV14	BV14	SK1	SK1	SK1
Date Collected:	9/9/84	20/2/84	20/2/84	29/2/84	1/82	12/83	1/83
Specimen Type:	A	A	A	A	C	C	C
Fatty Acid							
C10:0							
C12:0	2.41	2.01		6.13			
C14:0	3.95	10.95	0.12	12.27	4.02	12.79	3.31
C14:1w9	2.15						
C14:2							
C15:1	0.97						
C16:0	22.38	27.26	0.11		20.23	24.61	18.58
C16:1	8.51	21.53		41.19	17.49		15.91
C16:2	1.25						
C17:0	1.34						
C18:0	3.61	2.66			9.16	4.29	7.00
C18:1w9	21.03	35.60	0.53	11.06	34.66	30.17	33.93
C18:2w6	8.23		1.87	29.36	13.45	1.39	18.91
C18:3w3	4.35						2.36
C20:1w9	1.24						
C18:4							
C20:2							
C21:0							
C22:1w9							
C24:1w9							
C20:3	1.94						
C20:4			1.30				
C20:5w3	4.33						
C22:4							
C22:5w3							
C22:6w3							

Table 17 (continued)

Source:	SK1	SK1	SK1	SK5	SK5	SK5	SK5
Date Collected:	25/11/83	25/11/83	21/8/84	1/82	9/83	22/11/83	27/11/83
Specimen Type:	A	A	A	A	A	A	A
Fatty Acid							
C10:0			14.75				
C12:0		4.52				7.37	2.92
C14:0			1.99	3.74	3.68	6.33	
C14:1w9		2.10	4.65				
C14:2							
C15:1			2.00				
C16:0	21.18	26.15	14.75	19.52	19.81	34.25	35.88
C16:1	14.32	16.52	8.52	16.36	16.13	9.94	8.15
C16:2	2.37	2.29	3.29				
C17:0			3.64				
C18:0		7.35	2.95	10.71	7.61	6.89	11.96
C18:1w9	51.48	28.76	24.85	40.39	38.19	35.0	41.1
C18:2w6	10.65	6.25	11.45	8.48	13.1	0.21	
C18:3w3		2.15	0.70		0.88		
C20:1w9				0.16			
C18:4							
C20:2							
C21:0			0.14				
C22:1w9		2.30					
C24:1w9							
C20:3			1.88				
C20:4							
C20:5w3		1.61	2.51	0.63			
C22:4							
C22:5w3							
C22:6w3			1.41				

Table 17 (continued)

Source:	SK5	SK5	P4	P5	P1	P3	ARC
Date Collected:	27/11/83	9/1/84	1/8/84	10/7/84	29/5/84	29/5/84	
Specimen Type:	A	A	A	A	A	A	A
Fatty Acid							
C10:0		5.11	1.12	2.52	58.19	80.52	2.52
C12:0	3.49			2.09			
C14:0		1.29	8.38	3.10	0.46		4.04
C14:1w9		2.15		2.57	1.01		
C14:2							
C15:0		0.54		1.25			
C15:1		1.26	1.41	2.25	0.60	0.22	
C16:0	42.16	11.70		11.12	5.47	3.43	12.87
C16:1	6.18	10.37		8.21	4.66	2.02	11.38
C16:2		5.56	4.15	5.42	2.22	0.77	2.83
C17:0		3.16		2.97	0.72		
C18:0	8.89	3.25		4.78	1.04	0.72	2.98
C18:1w9	36.21	30.81	7.34	26.11	12.74	6.93	34.41
C18:2w6	3.07	18.64	4.65	11.33	5.19	1.81	10.44
C18:3w3		3.70	11.19	3.61	1.19	0.64	2.83
C20:1w9				0.94			
C21:0				1.64			
C22:0			1.85				
C22:1w9			1.72				
C20:3				1.48	1.18	0.47	
C20:5w3			11.71	4.09	2.34	1.22	5.85
C22:5w3			46.48				
C22:6w3		1.78		3.59	2.77	1.06	9.16

P1,P3, P4, P5 samples from experimental ponds
ARC sample freshly hatched from Artemia Reference Center cysts
A = Adult *Artemia*; C = cysts

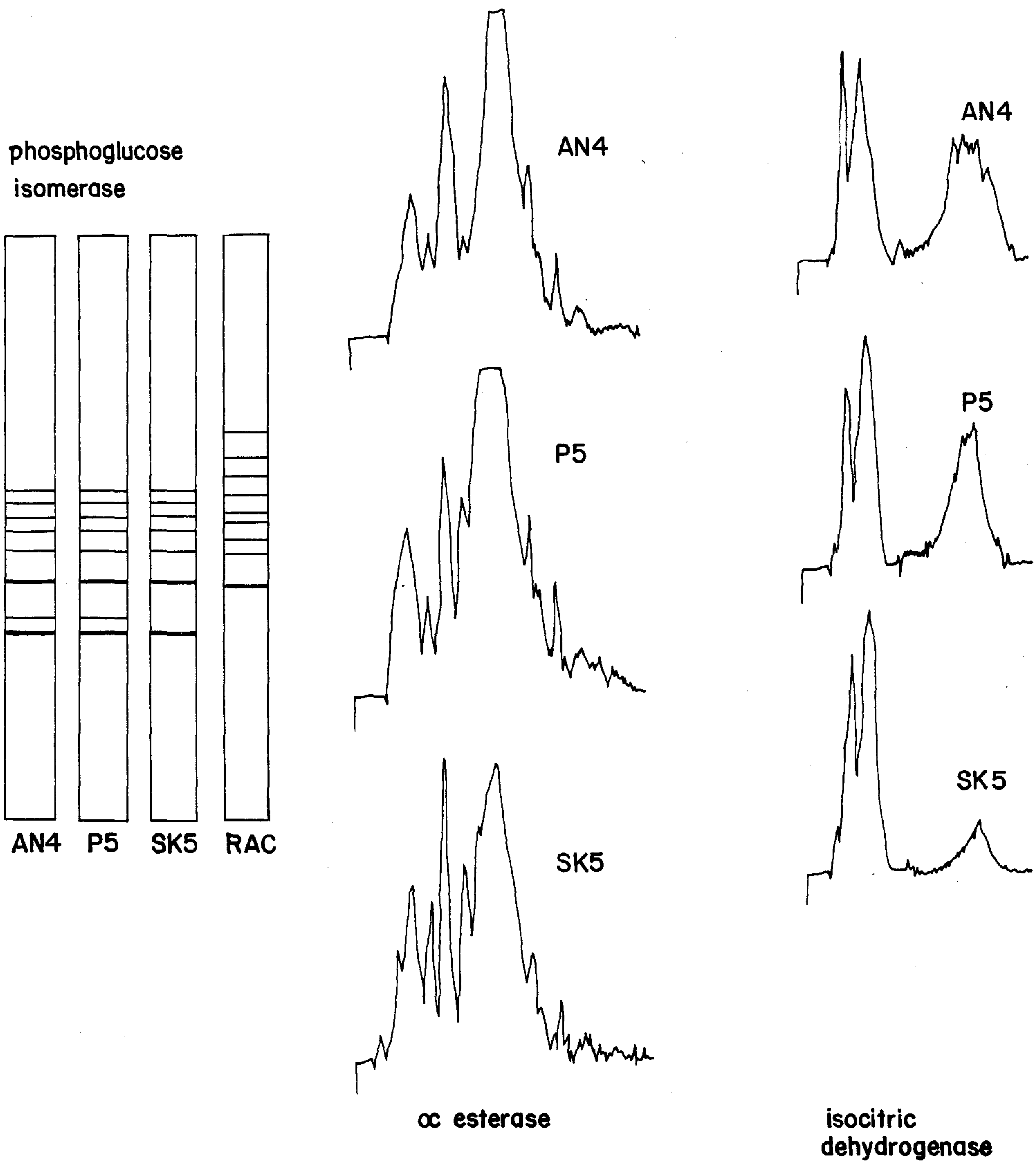
Table 17 (continued)

Source:	HSA	HSA
Date Collected:	1/9/84	1/9/84
Specimen Type:	---	---
Fatty Acid		
C10:0		6.93
C12:0		
C14:0	4.60	7.33
C14:1w9		
C14:2		
C15:1		3.24
C16:0	14.89	20.87
C16:1	4.40	8.18
C16:2	3.13	7.44
C17:0		
C18:0	3.42	3.44
C18:1w9	16.77	27.95
C18:2w6	3.62	
C18:3w3	8.98	10.93
C20:1w9		
C18:4		
C20:2		
C21:0		
C22:0	6.15	
C22:1w9		
C24:1w9		
C20:3		
C20:4		
C20:5w3	7.86	
C22:4		
C22:5w3		
C22:6w3	23.51	3.69

HSA = cultures of hypersaline algae derived from experimental ponds

Figure 22 -- Tracings of Densitometric Scans and Gel Isozyme Patterns of Artemia Samples

- i -



specimens do not appear to be significantly different from each other.

Samples from SK1, SK5,, and AN4 analyzed for chlorinated hydrocarbons had hexachlorobenzene concentrations of 2.0, 1.0, and 1.0 ppb respectively. Lindane, chloridane, PCB, and DDT concentrations in all samples were 0.5 ppb. These values are close to the limit of detection, and are not considered significant.

F. Investigation of Possible Manipulations to Optimize *Artemia* Production

Results of fertilization studies are summarized in Tables 18 and 19. Hypersaline algae responded to f/2 media, as well as to media enriched with 10% chicken manure, grain waste, or bagasse slurries. Nutrient analyses of these slurries are provided in Table 20. No increase in growth was observed in media enriched with phosphate alone, nor in cultures which received less than 1% waste slurry. The majority of algae which responded to enrichment were green flagellates of a size suitable for *Artemia* feeding. No response to fertilization was observed in samples innoculated with "mud".

Artemia reared on Tahitian *Isochrysis* did not survive to the end of the experiment, while average survival was 29% among *Artemia* fed on native hypersaline algae. Insufficient biomass was available for fatty acid analysis to confirm that the *Artemia* had the same PUFA profile as the algae upon which they had fed.

Following the introduction of normal seawater into the experimental ponds, marked stratification occured. The most striking consequence of this was the appearance of bottom water masses with temperatures exceeding 50 C, resulting from the insulating effect of an overlying freshwater lens. This stratification diminished over a period of four days. Within 12 hours of the introduction of seawater, the ponds developed masses of filamentous green algae on the bottom, in some cases on areas from which crystalline salt was still dissolving.

Observations following pond manipulation are summarized in Table 21. No increase in the proportion of cyst bearing females was noted, and reproductive mode appeared to remain relatively constant during 14 days following the introduction of *Artemia* to the manipulated ponds.

Table 18: Algal Growth in Media Enriched with Various Materials

Medium	Maximum Cell Density ('0,000 cells/ml)	Ammonia Nitrogen (ug-at/l)
Pond Water	7.5	32
Pond Water + Press Mud		
10%	13	32.62
1%	32	32.062
0.1%	14	32.006
0.01%	6.7	32.001
Pond Water + Chicken Manure		
10%	116	84.3
1%	18	37.23
0.1%	23.3	32.523
0.01%	4.2	32.052
Pond Water + Grain Waste		
10%	125	288.6
1%	22	57.66
0.1%	4.2	36.2
0.01%	1.8	32.257
Pond Water + Bagasse		
10%	56	57.4
1%	7	34.54
0.1%	5.5	32.254
0.01%	4.2	32.025
Pond Water + NPK	6.7	---
f/2	174	---

Table 19: Algal Cell Density in Various Media Innoculated with Pond Mud

Medium	Maximum Cell Density ('0,000 cells/ml) Day 1	Day 14
Pond Water	20.8	10
Pond Water + Press Mud	19.6	20
Pond Water + Chicken Manure	19.6	17.5
Pond Water + Grain Waste	12.1	11.3
Pond Water + Bagasse	12.5	6.3
Pond Water + NPK	6.7	2.5
f/2	17.1	7.5

Table 20: Analyses of Nutrient Content of Various Waste Products (ug-at/l)

Waste Product	Nitrate+Nitrite Nitrogen	Nitrite Nitrogen	Ammonia Nitrogen	Phosphate
Press Mud	2.6	ND	6.3	54.3
Chicken Manure	7.1	0.2	521.9	201.4
Grain Waste	2.0	0.1	2566.4	64.3
Bagasse	2.0	ND	253.5	71.4

Table 21: Summary of Observations Following Manipulation of Experimental Ponds

Date	Salinity	%M	%F	%J	%CB	%LB	%NR	Miscellaneous
18/8/84								
Pond 1	167							
Pond 2	120							
Pond 3	162							
Pond 4	141							
Pond 5	144							
Pond 6	204							
20/8/84								
Pond 2		42	22	36	0	47	53	
Pond 3		41	24	35	0	11	89	
Pond 5		38	38	24	0	0	100	
Pond 6		59	12	29	0	0	100	
22/8/84								
Pond 2		58	42	0	0	36	64	
Pond 3		25	75	0	0	100	0	*
Pond 4		80	20	0	0	0	100	*
Pond 5		0	33	67	0	0	100	*
Pond 6		50	50	0	0	0	100	*
24/8/84								
Pond 2		41	53	6	0	78	22	
Pond 3		44	56	0	0	93	7	

Table 21: (continued)

Date	Salinity	%M	%F	%J	%CB	%LB	%NR	Miscellaneous
25/8/84								
Pond 1	154							
Pond 2	245							
Pond 3	256							
Pond 4	157							
Pond 5	260							
Pond 6	192							
27/8/84								
Pond 2		40	49	11	9	91	0	
Pond 3		14	79	7	9	91	0	
Pond 4		53	39	8	30	70	0	
Pond 5		33	31	36	33	50	17	
Pond 6		37	49	15	5	95	0	
28/8/84								
Pond 1								diss. oxygen 2.2 - 2.6 ppm
Pond 2								diss. oxygen 0 - 2.2 ppm
Pond 3								diss. oxygen 0.6 - 2.6 ppm
Pond 4								diss. oxygen 3.6 - 5.6 ppm
Pond 5								diss. oxygen 2.0 - 2.2 ppm
Pond 6								diss. oxygen 0 - 2.6 ppm
29/8/84								
Pond 1								B.O.D. = 1.8 mg/l/day
Pond 2								B.O.D. = 0.4 mg/l/day
Pond 3								B.O.D. = 0.7 mg/l/day
Pond 4								B.O.D. = 0.6 mg/l/day
Pond 5								B.O.D. = 1.1 mg/l/day
Pond 6								B.O.D. = 1.6 mg/l/day

Table 21: (continued)

Date	Salinity	%M	%F	%J	%CB	%LB	%NR	Miscellaneous
31/8/84								
Pond 1	153	16	45	39	0	100	0	
Pond 2	235	43	50	7	14	86	0	
Pond 3	250	26	62	12	5	95	0	
Pond 4	149	26	37	37	0	90	10	
Pond 5	---	41	45	14	20	80	0	
Pond 6	181	32	45	23	10	90	0	

%M -- Percent of males in sample
%F -- Percent of females in sample
%J -- Percent of juveniles in sample
%CB -- Percent of females bearing cysts
%LB -- Percent of females bearing live offspring
%NR -- Percent of non--reproductively active females
* -- sample size less than 10 Artemia

IV. DISCUSSION

A. Evaluation of Principal Hypotheses

1. "Artemia are endemic to Eastern Caribbean salt ponds and when reared on phytoplankton cultures derived from the same ponds have nutritional value which is at least equal to reference Artemia reared on standard diets."

Data presented in Table 2 establish that Artemia are endemic to 25% of the ponds and 56% of the islands surveyed. Direct evaluation of the second part of this hypothesis was hampered by difficulties encountered with isolation and culture of native salt pond phytoplankton. It is likely that many of the Artemia populations investigated in this study derive a major portion of their nutrition from detritus, bacteria, and dissolved or suspended organic particles rather than phytoplankton specifically. This appears to be the case in natural brine shrimp populations in other locations as well (Sorgeloos, 1980).

Concentration of polyunsaturated long-chain fatty acids (PUFA) was used as an index of nutritional value. Many animals including marine fishes, penaeid shrimps, and oysters have a dietary requirement for these fatty acids; hence this is a useful measure of the potential value of various Artemia strains for mariculture purposes. Most samples of cysts and adult Artemia collected during preliminary surveys and stored in formalin or at -5 C were virtually devoid of long-chain fatty acids. These findings resulted in concern as to possible degradation of samples prior to analysis (though this storage has appeared adequate in previous work). Subsequent samples were extracted in the field as a precaution, and most of the latter samples had significant quantities of long-chain PUFA. In view of recent work showing minimal enzymatic or oxidative degradation of Artemia lipids under storage (Sasaki and Capuzzo, 1984) it seems unlikely that significant degradation actually occured in the early samples, but this possibility requires further investigation.

An alternative explanation for the low concentration of long-chain PUFA in early samples is that the nutritive materials which were available to the brine shrimp during the spring/summer were different that those available during fall/winter. It is interesting to note that the only early samples of endemic Artemia with significant concentrations of long chain polyunsaturated fatty acids were collected from ponds having salinity less than 90 o/oo. Such moderate salinities were unusual in Artemia-containing ponds visited during the study. Lower salinities are apt to be more favorable to phytoplankton growth in general, and it is possible that these algae play a more important role in the nutrition of *Artemia* living under moderate salinity conditions.

If these results do indicate a seasonal periodicity in the fatty acid composition of *Artemia*, endemic brine shrimp populations may be of limited nutritional value under certain natural conditions. But such conditions may be modified to improve the content of long chain PUFA in native brine shrimp as discussed below.

A principal concern in this investigation is possible manipulations to optimize *Artemia* quality and production in endemic salt ponds. The nutritional composition of San Francisco Bay *Artemia*, and hence its dietary value, can be modified through various feeding regimes (e.g., Watanabe, 1980; Sakamoto *et al*., 1982). This suggests that the nutritional value of *Artemia* endemic to the Eastern Caribbean might be similarly modified in salt pond culture. Several common algal species including *Chlorella* (Watanabe *et al*, 1980) and *Chaetocerus* (Sakamoto *et al*, 1982) are known to produce favorable concentrations of PUFA when fed to *Artemia*, but it is desirable that pond manipulations utilize endemic algal or other nutritive species (e.g., bacteria) in preference to introducing non-native organisms.

Fatty acid profiles of algae cultured from hypersaline ponds indicate that the algae tested are promising as food sources with potential for improving the concentration of long-chain PUFA in *Artemia*. Natural "blooms" of these algae are probably prevented by low nutrient concentrations in the ponds which could be modified by means of appropriate fertilization. *In vitro* fertilization experiments suggest that chicken manure, grain waste, and bagasse are locally available and potentially useful for this purpose. It is probably significant that the waste products which enhanced algal growth were high in ammonia nitrogen (cell density, in fact, appears directly related to the ammonia concentration). Phosphate did not appear to be directly limiting, and enrichment with phosphate alone did not enhance algal productivity *in vitro*.

The use of waste products as algal fertilizers has attracted considerable attention as a way of reducing costs in attaining higher yields in aquaculture (e.g., Edwards, 1980; Wohlfarth and Schroeder, 1979; Mann and Ryther, 1977; Ryther, 1981). The tropics are generally well-suited to the use of a variety of wastes due to accelerated microbial remineralization of organic material in elevated temperatures (Benefield and Randall, 1980).

Waste products applicable to pond fertilization in aquaculture may be animal or agro-industrial in origin. Several such products are frequently available on smaller Caribbean islands. Sugar cane is is still grown on several islands. When sugar cane is crushed at the mills it is separated into two major components, cane juice and bagasse. Cane juice is converted to sugar and molasses, the residue being referred to as "press mud". Distilleries and breweries are common. Domestic animals such as pigs, goats, and cows are common components of village-based rural economies, along with occasional dairies and egg farms.

Avault *et al*. (1978) tested bagasse, press mud, and chicken manure in earthen ponds as part of an experimental crawfish culture project. Yields were 500 kg/ha for chicken manure, 400 kg/ha for press mud cake, and 200 kg/ha for bagasse. Heavy blooms of algae were noted. Nugent (1978) reported a yield of 2500 kg/ha/yr of *Tilapia* from ponds fertilized with brewery wastes. Sorgeloos (1980, 1982) suggests that these sorts of organic fertilizers, perhaps in combination with supplementary inorganic fertilizer, may be effective in the production of *Artemia*.

Bacterial oxygen consumption may also be enhanced by fertilizers, and this may constrain their use in hypersaline ponds. Wohlfarth and Schroeder (1979) found that once-yearly applications of large amounts of fertilizer resulted in depletion of oxygen and fish kills. This effect was reduced when smaller amounts of fertilizer were added at more frequent intervals. Kohler and Pagan-Font (1978) report dawn dissolved oxygen levels ranging from 0.5 to 5.8 ppm in fertilized ponds, compared to 6.7 ppm in controls. These effects may be particularly serious in salt ponds which are inherently oxygen-poor due to the low solubility of oxygen in high temperature hypersaline waters.

2. "The reproductive mode of Eastern Caribbean *Artemia* will vary when reared at different levels of temperature, salinity, and dissolved oxygen."

Pond salinity and reproductive mode data presented in Figures 16 - 21 suggest a correlation between sudden changes in salinity and increased production of cysts vs live offspring. Laboratory studies on Christmas Island have shown that cyst production can be induced when sexually mature *Artemia* are transferred from water of 35 o/oo to media of 70 o/oo. After two or three generations, production reverted to ovoviviparity. A similar experience occured in the commercial *Artemia* production facility at Macau, Brazil (Sorgeloos, *personal* communication).

The relationship between salinity and reproductive mode in Eastern Caribbean *Artemia* is different. It has been previously noted (Kristensen and Hulscher-Eweis, 1972) that while brine shrimp in northern salt ponds produce live offspring at low salinities and cysts at higher salinities, Antillean strains are viviparous for the most part in salinities above 85 o/oo and oviparous in less concentrated media. The same authors note that when brines of high density have been recently diluted, viviparity still occurs. *Artemia* populations studied in St. Kitts, in contrast, showed an increase in the proportion of cyst-producers two to four days after sudden drops (usually 10 o/oo or more) in salinity during a 24 hour period. Versichele and Sorgeloos (1980) state that *Artemia* alter reproductive mode two to three reproductive cycles after exposure to stress (in this case, reduced oxygen tension). This observation makes it less certain that reduced salinity caused an increase in cyst production in experimental ponds, as the observed "response" occured in a much shorter period of time. On the other hand, St. Kitts *Artemia* have been shown to be physiologically distinct from other strains, and observations on the latter may not be applicable. Moreover, reduced salinity experiments produced a significant increase in cyst production in experiments at the University of the West Indies.

It is clear that the physiological mechanisms which determine reproductive mode do not operate in the same manner among all *Artemia* strains. It also seems unlikely that a single environmental factor serves as a "trigger" for cyst production. Thus, while salinity appears to be the factor which induced oviparity in St. Kitts *Artemia*, cyclic oxygen depletion has been shown to result in a shift from ovoviviparity to ovoviparity when San Francisco Bay *Artemia* are reared at 95 o/oo *in vitro* (Versichele and Sorgeloos, 1980). In the latter case it was noted that low oxygen tensions stimulate hemoglobin synthesis, and that cyst formation is enhanced by the addition of chelated iron (which also stimulates hemoglobin synthesis) to the culture medium (cyst shells are usually impregnated with a hematin-like substance). Similar correlations have been noted between cyst production and food quality or quantity (Baker, 1966; D'Agostino and Provasoli, 1968; Dutrieu, 1960).

Interrelationships between physical and biological factors make it impossible to attribute the response of Artemia to change in a specific parameter. Algal composition in the ponds, for example, may be altered significantly by sudden changes in salinity. Lower oxygen tension may indirectly have affected St. Kitts brine shrimp: Reduced salinity would raise the saturation tension of oxygen in pond water, and a subsequent rise in salinity would cause dissolved oxygen to be reduced. At this point the best interpretation of the reported results is that Eastern Caribbean Artemia respond to sudden changes in their environment by producing a greater proportion of cysts than live offspring.

From a manipulative standpoint, salinity is considerably easier to control than dissolved oxygen in ponds. The ability to regulate reproductive mode has potential importance in small-scale Artemia mariculture, as it allows the culturist to tailor the form of his product in response to market demand. Soluble iron was present in very low levels in the ponds studied; addition of chelated iron may be a further means for promoting cyst production, though it would probably be more difficult to reverse than salinity changes.

3. "The size of Artemia populations and reproductive mode in Eastern Caribbean species will vary with changes in temperature, salinity, dissolved oxygen, nitrates, nitrites, ammonia, or phosphate."

Data presented in Figures 16 - 21 indicate a negative correlation between population size and salinity in experimental ponds. Laboratory studies indicated that temperature and salinity have significant effects on the survival of Reference Artemia over the range tested. St. Kitts Artemia, while affected by temperature, did not show significant response to salinity, probably because the upper limit of their salinity tolerance is well above the range examined. Laboratory and pond studies indicate that optimum salinity and temperature conditions for St. Kitts Artemia lie in the range 150 - 180 o/oo, 25 - 32 C.

Maximum population size in experimental pond populations was obtained at salinities ranging from 160 - 185 o/oo (except in the case of pond 2 in which maximum population size was obtained at 220 o/oo). Reported estimates of population size (Persoone and Sorgeloos, 1980) in various Artemia habitats range from 250 (in San Francisco Bay salt ponds) to 150,000 (Crimea salt lakes) Artemia per square meter (assuming that one adult Artemia has a wet weight of approximately 2 mg). Reported density of *Artemia* in the Great Salt Lake is 10 *Artemia* per liter, while a density of 500 adult *Artemia* per liter has been considered adequate for low technology brine shrimp culture suitable for use by commercial prawn hatcheries (Dwivedi et al, 1980). These figures compare with an estimated maximum density of 3900 *Artemia* per square meter (approximately 130 per liter) in experimental ponds in St. Kitts. Natural pond production in St. Kitts compares favorably with that of other locales, but requires enhancement to equal the production of intensive culture facilities.

Dissolved oxygen at salinities in excess of 260 o/oo seldom exceeded 1.0 ppm, and may have been significant to the decline of brine shrimp exposed to extremely hypersaline conditions. Reference *Artemia* were more sensitive to high levels of temperature and salinity than brine shrimp from St. Kitts. Because of the apparently minor role of algae in the nutrition of *Artemia* in the salt ponds under study and the time required for replicate analyses, an extensive investigation of nitrogen- and phosphorus-containing nutrients was not undertaken. Nutrient analyses of water samples taken before and after experimental salinity manipulation show a marked decrease in ammonia nitrogen following the introduction of seawater. Nitrate nitrogen ranged from 0.1 to 1.0 ug-at/l before manipulation, and was generally undetectable afterward. Phosphate was generally undetectable before or after manipulation.

4. "Natural salt ponds may be modified to produce levels of factors tested in Hypothesis 3 which optimize production of *Artemia* cysts.

At the time scheduled for pond manipulation, there were no clear indications of the relative value of endemic algae, nor of fertilization procedures to which useful algae might respond, nor of laboratory-determined optima for biomass or cyst production. Preliminary analyses of physical factor and reproductive mode data from experimental ponds suggested that lowered salinity was correlated with increased cyst production. To further test this possibility and to obtain direct experience with the sort of manipulation which might be used in a small-scale *Artemia* culture operation, salinity in the experimental ponds was lowered as described in section II. No increase was observed in the frequency of ovoviparity within 14 days following this manipulation, after which field studies were terminated.

While these results do not disprove the null hypothesis, this limited experience clearly does not suggest that other manipulations would be similarly ineffective. Artemia in the experimental ponds were not well-acclimated to these habitats prior to alteration of salinity, and it is quite possible that a similar manipulation of established populations would have produced the anticipated result.

Fertilization of ponds has considerable potential, and information obtained subsequent to the field studies provides a starting point for such manipulation. As noted above, ammonia nitrogen appears to be the primary nutrient factor limiting the growth of algae with good concentrations of long chain PUFA. Algal growth was directly proportional to ammonia nitrogen concentration over the range 25 - 256 ug-at/l ammonia nitrogen. Concentrations of 2.5 ug-at/l did not have clear value. Grain waste is particularly rich in ammonia nitrogen. Effects of these ammonia concentrations on Artemia should be evaluated prior to actual pond fertilization.

B. Evaluation of Development Potential for Artemia Culture in the Eastern Caribbean

The technical investigations which comprise this study indicate that (1) useful quantities of Artemia can be produced in salt ponds endemic to the Eastern Caribbean, (2) the fatty acid composition of some populations of endemic Artemia is favorable for culture of other species; (3) some strains of algae isolated from Eastern Caribbean salt ponds have fatty acid compositions favorable to the production of nutritionally valuable Artemia; and (4) the reproductive mode of endemic Artemia strains is correlated with changes in salinity, and these changes may provide means for some degree of control over the production of cysts vs live offspring.

Evaluation of the potential for brine shrimp culture in Eastern Caribbean salt ponds involves a number of other considerations. These have been summarized by Pillay (1974) and Matsuda (1978), and provide a framework for this evaluation.

1. Economic Feasibility

a. Investment criteria

(1) commercial -- In many cases the principal consideration relates to the value of a salt pond compared to the value of tourist developments or marinas which might

use the same area were the salt pond to be filled or dredged. The potential for *Artemia* culture in iself is probably insufficient basis for an adequate case for salt pond preservation. If there is no such competing use, a project based on simple modifications may begin with very little capital investment. Because *Artemia* are normally highly fecund with rapid growth and maturation, initial investments might be quickly repaid. Small operations may conceivably be expanded as evaluations of manipulative options are completed. A scaled operation of this sort allows feasibility assessments to be made at each stage, and provides the option to delay expansion without detracting from the viability of earlier activities.

(2) social -- Once established, much of the work involved with *Artemia* culture requires relatively little technical capability. Consequently there are good prospects for local employment, particularly if non-mechanized harvest procedures are used. It is conceivable that technical assistance could be provided for the establishment of small-scale brine shrimp culture operations which could be managed entirely by unskilled personnel, though some ongoing technical input would be desirable for optimum production. Since the primary market for *Artemia* in the Eastern Caribbean is for shrimp food or for export to pet stores, no substantial direct impact on local nutrition is expected. Foreign exchange generated by export of processed cysts or adult brine shrimp could be useful in solving problems with balance of payments which plague most Eastern Caribbean countries.

b. Supply and demand

(1) consumer acceptability and preferences -- Vanhaecke and Sorgeloos (1983) suggest that the primary criteria for selecting a specific cyst strain are price and hatching quality; if these are comparable, strains producing large nauplii are preferred. Penaeid shrimp culturists currently operating in the Eastern Caribbean, on the other hand, are more concerned with reliable supply of substantial quantities of live biomass; the relative nutritional value is of secondary interest. This view may change as these operations become more established, but the present market for *Artemia* within the region seems less particular than is the case elsewhere. Expansion to markets outside the Eastern Caribbean would necessitate greater attention to the criteria listed above. Production of cysts in addition to or instead of live biomass would require close attention to cyst quality and packaging in order for the product to be acceptable. *Artemia* biomass is sold live or frozen to aquarium pet shops, a market which

consumes 50% of the total (45,000 lb in 1980) production from the Great Salt Lake. Beyond this traditional use of *Artemia* biomass, recent experiments have indicated further potential in both aquaculture and husbandry of terrestrial animals. *Artemia* production can be integrated in local aquaculture developments, thus eliminating factors involved in cyst production, harvesting, processing and hatching, and obviating the expense of cyst importation. In Brazil, experiments have been successfully conducted using dried *Artemia* as an inexpensive substitute for fish meal in the formulation of pellets to feed shrimp in grow-out ponds.

(2) competing products -- Commercial mariculture feeds are not locally produced and are expensive to import. *Artemia* are a principal ingredient of many feeds, and when available are often used to supplement or replace commercial feeds.

(3) total requirements for domestic and export consumption -- Absolute demand is difficult to assess. Recent estimates suggest that the world demand is at least twice the current supply. Increased attention is being directed toward the use of *Artemia* in poultry and cattle feeds (Corazza and Saylor, 1983), and aquaculture activity is expanding throughout the Caribbean. Costs of feed importation often constitute the greatest single expense in animal husbandry. These trends suggest that the demand for brine shrimp is likely to at least equal the production which could be expected from small-scale *Artemia* production in local salt ponds.

(4) other sources of supply -- Brine shrimp are produced throughout the world. Costs of transportation coupled with increased cost of intensive *Artemia* culture suggest that a locally produced product will be able to compete effectively with other producers in local (and possibly international) markets. International supplies of *Artemia* cysts have remained relatively static since 1979 when world wide production was estimated at 100 metric tons (Sorgeloos, 1982). Improved techniques for *Artemia* culture (largely due to the work of the Artemia Reference Center at the Laboratory for Mariculture, Ghent, Belgium) have prompted increased activity in *Artemia* production. Much of the resultant product appears to be sold directly to consumers, primarily in the far east, and has not appreciably affected the world market.

(5) demand elasticity -- Local markets will vary according to the success of other aquaculture operations. The high-risk nature of many of these ventures makes it desirable that alternative local markets (e.g.,

animal feeds) be developed as well. There has been no recent indication of an overall decline in world demand.

c. Investment analysis

Investment and operating costs, cash flow projections, and financial profitability depend upon the size of operation contemplated and the degree of developmental research which will be undertaken. Ideally, a pilot project should be established to build local competence in production procedures while simultaneously conducting comparative investigations to determine optimum production techniques. Low labor and technology requirements coupled with a substantial market demand should form the basis for a favorable financial picture. The direct impact on the national economy probably would not be substantial, but indirect benefits in terms of employment and reduction of imports should also be considered.

Investment analyses of two hypothetical commercial *Artemia* culture scenarios are presented in Tables 22 - 25. The variability displayed in the data reported above is indicative of the degree of uncertainty inherent in making this sort of projection. Alteration of any of the primary assumptions can drastically alter the entire picture, but these analyses are presented to suggest that commercial production of *Artemia* in Eastern Caribbean salt ponds can be feasible under conditions which can reasonably be assumed to occur in these ponds. A 20 ha pond 100 m from the sea is assumed for all analyses.

All models are based on a combined production of live *Artemia* biomass (which is frozen prior to marketing) and cysts. As a starting point, biomass production figures are based on the average standing biomass in the six experimental ponds observed in this study during the period in which salinity ranged from 160 - 200 o/oo; 190,000,000 *Artemia*/ha. If an adult brine shrimp weighs 2 mg wet, the average biomass is equivalent to 380 kg/ha. *Artemia* production is sufficient to permit harvesting 99% of this biomass every three weeks under the following conditions: time to maturity = 12 days; interval between broods = 5 days; total number of broods per female = 4; sex ratio = 1:1; average number of eggs per brood = 40 (reproductive capacity determined for St. Kitts *Artemia* at the Univesity of the West Indies is about half this value, but the conditions under which these determinations were made are considered marginal considering *in situ* observations made after the laboratory work was done; the figure used was reported as an average by Dwivedi *et al*. (1980), and is considered more likely to be representative of Eastern

Caribbean *Artemia* under favorable conditions). To aid in assessing the viability of specific brine shrimp culture projects, a computer program has been developed which models *Artemia* production given different values for these parameters.

Prices for live or frozen *Artemia* biomass are difficult to determine. Dry commercial feed for the St. Kitts shrimp farm costs $0.55/kg. Assuming a wet:dry weight ratio of 9:1, wet *Artemia* would have to cost $0.055/kg to be competitive. Pet shops, on the other hand, often sell frozen *Artemia* at prices equivalent to over $4.00/kg. In the analyses reported here, the sale price for frozen *Artemia* has been calculated as that required to break even and repay capital investment after a period of five years. A second analysis has been prepared for each scenario using more favorable cyst production figures and lower sale price for frozen biomass. In some cases, the latter figures approach the price needed to attract local shrimp farmers. These analyses also reflect the effect of parameter changes on the economic analyses; it is essential that reasonable values be established for these parameters on a case-by-case basis.

Vos *et al*. (1984) report that cyst production in manipulated salt ponds ranged from 10 to 60 kg/ha over a five month period, while Persoone and Sorgeloos (1980) give a figure of 10 to 20 kg/ha per season for good *Artemia* biotopes (which includes temperate salt ponds having negligible numbers of brine shrimp for part of the year). Prices for cysts have fluctuated over recent years, having fallen from very high levels in the late 1970's. Average prices were reportedly $20 - $40 per kilogram in 1980, $30 - $67/kg in 1982, and $67 - $71/kg in 1984. Prices also vary depending upon the commercial source. A figure of$69/kg has been used here.

The first scenario represents what is considered the minimum investment, and manipulation which extends only to pumping seawater to maintain or alter pond salinity. Cyst production is estimated at 10 kg/ha/yr, and biomass at 380 kg/ha/4 wk. To break even after five years, a sale price of $0.31/kg is required. The net present value after 10 years is $6,900 at a 10% discount rate, or $-5,000 at a 20% discount rate. The internal rate of return is estimated to be 15%.

An alternative analysis for the first scenario is based on a production figure for cysts of 20 kg/ha/yr, and for biomass of 380 kg/ha/2 wk. If frozen biomass is sold at $0.10/kg, the business in the second analysis breaks

Table 22: Financial Projection for Hypothetical *Artemia* Culture Operation -- Scenario 1; Minimal Investment, Conservative Production Estimates

	Year 1	Year 2	Year 3	Year 4	Year 5
Production					
Biomass (kg)	98800	98800	98800	98800	98800
Cysts (kg)	200	200	200	200	200
Revenue					
Biomass ($0.31/kg)	30628	30628	30628	30628	30628
Cysts ($69/kg)	13800	13800	13800	13800	13800
Total	44428	44428	44428	44428	44428
Capital Costs					
Pumps & Hose	5000				
Harvesting Equipm.	5000				
Freezers	10000				
Vehicle	10000				
subtotal	30000	0	0	0	0
Operating Costs					
Salaries	17800	17800	17800	17800	17800
Lease	4004	4004	4004	4004	4004
Fuel	4888	4888	4888	4888	4888
Maintenance	2600	2600	2600	2600	2600
Insurance	520	520	520	520	520
Legal/accounting	2600	2600	2600	2600	2600
Utilities	1560	1560	1560	1560	1560
Nets, etc.	4472	4472	4472	4472	4472
subtotal	38444	38444	38444	38444	38444
Total Outflows	68444	38444	38444	38444	38444
Period Cashflow	-24016	5984	5984	5984	5984
Cumulative Cashflow	-24016	-18032	-12048	-6064	-80

Cumulative Cashflow at end of Year 6 = 5904
Cumulative Cashflow at end of Year 7 = 11888
Cumulative Cashflow at end of Year 8 = 17872
Cumulative Cashflow at end of Year 9 = 23856
Cumulative Cashflow at end of Year 10 = 29840

Table 23: Financial Projection for Hypothetical *Artemia* Culture Operation -- Scenario 1; Minimal Investment, More Optimistic Production Estimates

	Year 1	Year 2	Year 3	Year 4	Year 5
Production					
Biomass (kg)	197600	197600	197600	197600	197600
Cysts (kg)	400	400	400	400	400
Revenue					
Biomass ($0.10/kg)	19760	19760	19760	19760	19760
Cysts ($69/kg)	27600	27600	27600	27600	27600
Total	47360	47360	47360	47360	47360
Capital Costs					
Pumps & Hose	5000				
Harvesting Equipm.	5000				
Freezers	10000				
Vehicle	10000				
subtotal	30000	0	0	0	0
Operating Costs					
Salaries	17800	17800	17800	17800	17800
Lease	4004	4004	4004	4004	4004
Fuel	4888	4888	4888	4888	4888
Maintenance	2600	2600	2600	2600	2600
Insurance	520	520	520	520	520
Legal/accounting	2600	2600	2600	2600	2600
Utilities	1560	1560	1560	1560	1560
Nets, etc.	4472	4472	4472	4472	4472
subtotal	38444	38444	38444	38444	38444
Total Outflows	68444	38444	38444	38444	38444
Period Cashflow	-21084	8916	8916	8916	8916
Cumulative Cashflow	-21084	-12168	-3252	5664	14580

Cumulative Cashflow at end of Year 6 = 23496
Cumulative Cashflow at end of Year 7 = 32412
Cumulative Cashflow at end of Year 8 = 41328
Cumulative Cashflow at end of Year 9 = 50244
Cumulative Cashflow at end of Year 10 = 59160

Table 24: Financial Projection for Hypothetical *Artemia* Culture Operation -- Scenario 2; More Extensive Investment, Conservative Production Estimates

	Year 1	Year 2	Year 3	Year 4	Year 5
Production					
Biomass (kg)	790400	790400	790400	790400	790400
Cysts (kg)	700	700	700	700	700
Revenue					
Biomass ($0.118/kg)	93267	93267	93267	93267	93267
Cysts ($69/kg)	48300	48300	48300	48300	48300
Total	141567	141567	141567	141567	141567
Capital Costs					
Pumps & Hose	6145				
Harvesting Equipm.	4250				
Freezers	10000				
Vehicle	10000				
Building	16000				
Levees	15000				
Dredging	33400	18400	18400	5200	0
Generators	4000				
Misc.	2000	500	500	500	500
subtotal	100795	18900	18900	5700	500
Operating Costs					
Lease	7500	7500	7500	7500	7500
Harvest Labor	15600	15600	15600	15600	15600
Personnel	54400	54400	54400	54400	54400
Packaging/Distrib.	10400	10400	10400	10400	10400
Fertilizer	1040	1040	1040	1040	1040
Fuel	12324	12324	12324	12324	12324
Maintenance	5200	5200	5200	5200	5200
Insurance	2330	2330	2330	2330	2330
Legal/Accounting	3000	3000	3000	3000	3000
Administration	1000	1000	1000	1000	1000
subtotal	112794	112794	112794	112794	112794
Total Outflows	213589	131694	131694	118494	113294
Period Cashflow	-72022	9873	9873	23073	28273
Cumulative Cashflow	-72022	-62149	-52276	-29203	-930

Cumulative Cashflow at end of Year 6 = 27343
Cumulative Cashflow at end of Year 7 = 55616
Cumulative Cashflow at end of Year 8 = 83889
Cumulative Cashflow at end of Year 9 = 112162
Cumulative Cashflow at end of Year 10 = 140435

Table 24 (continued)

Assumptions:

5,000 ft of levees required at $5/ft
1,072.5 ft of piping needed at $2/ft
Building is 20 x 40 ft at $20/sq ft
Two generators required at $2,000 each
Two diesel pumps required at $2,000 each
Dredging costs $15,000 for materials and $3,000/ha dredged; at 7.5 weeks/ha, 151 weeks required for job; 46 operative weeks/yr
Packaging and distribution costs are $200/wk
Personnel includes all employees except harvest labor:

Manager	20,000
Technical Adviser	10,000
Mechanic	10,400
Driver	8,000
Secretary/bookkeeper	6,000
	(annual wages)

Insurance includes $80 on building, $200 on contents, $300 on vehicle, 2.5% of all wages and salaries
Fuel costs include $56/wk for truck and $181/wk for generators
Fertilizer is $20/wk
Administrative costs include office supplies and advertising
Repairs and maintenance costs are $100/wk

Table 25: Financial Projection for Hypothetical *Artemia* Culture Operation -- Scenario 2; More Extensive Investment, More Optimistic Production Estimates

	Year 1	Year 2	Year 3	Year 4	Year 5
Production					
Biomass (kg)	790400	790400	790400	790400	790400
Cysts (kg)	1400	1400	1400	1400	1400
Revenue					
Biomass ($0.08/kg)	63232	63232	63232	63232	63232
Cysts ($69/kg)	96600	96600	96600	96600	96600
Total	159832	159832	159832	159832	159832
Capital Costs					
Pumps & Hose	6145				
Harvesting Equipm.	4250				
Freezers	10000				
Vehicle	10000				
Building	16000				
Levees	15000				
Dredging	33400	18400	18400	5200	0
Generators	4000				
Misc.	2000	500	500	500	500
subtotal	100795	18900	18900	5700	500
Operating Costs					
Lease	7500	7500	7500	7500	7500
Harvest Labor	15600	15600	15600	15600	15600
Personnel	54400	54400	54400	54400	54400
Packaging/Distrib.	10400	10400	10400	10400	10400
Fertilizer	1040	1040	1040	1040	1040
Fuel	12324	12324	12324	12324	12324
Maintenance	5200	5200	5200	5200	5200
Insurance	2330	2330	2330	2330	2330
Legal/Accounting	3000	3000	3000	3000	3000
Administration	1000	1000	1000	1000	1000
subtotal	112794	112794	112794	112794	112794
Total Outflows	213589	131694	131694	118494	113294
Period Cashflow	-53757	28138	28138	41338	46538
Cumulative Cashflow	-53757	-25619	2519	43857	90395

Cumulative Cashflow at end of Year 6 = 136933
Cumulative Cashflow at end of Year 7 = 183471
Cumulative Cashflow at end of Year 8 = 230009
Cumulative Cashflow at end of Year 9 = 276547
Cumulative Cashflow at end of Year 10 = 323085

even during the fourth year. The net present value after 10 years is $21,800 at a 10% discount rate, or $7,400 at 20%. The internal rate of return is estimated at 25%.

The second scenario involves greater investment and includes provision for dredging ponds to increase depth (and consequently increase available water volume and reduce ambient water temperature. In order to be worthwhile, such manipulations should increase biomass production by a factor of at least four (as a result of incresed pond volume through dredging and increased food availability as a result of fertilization); biomass production is set at 1520 kg/ha/2 wk, while cyst production is assumed to be 35 kg/ha/yr. On this basis, a sale price of $0.118/kg is required for frozen biomass to break even after five years. The net present value after 10 years is $46,800 at a 10% discount rate, or $-3,000 at 20%. The internal rate of return is estimated at 19%.

If cyst production can be increased to 70 kg/ha/yr, and biomass is sold at $0.08/kg, the business breaks even in the third year. The net present value after 10 years is $76,800 at a 20% discount rate, or $33,400 at 30%. The internal rate of return is estimated to be about 40%.

2. Environmental Stability

a. Land availability

Most salt ponds, whether publically or privately owned, are considered undeveloped property. As such, they are generally available for purchase. But because most owners place very high value on their property in anticipation of sale to real estate or marina developers, purchase is not a realistic option for brine shrimp culture projects. Leasing is the most viable option if land is privately held. If dredging or filling is imminent, it is highly important that endemic algae and <u>Artemia</u> strains be isolated and maintained in culture as a stop-gap means of preserving genetic diversity. Though beyond the scope of this report, it is critical to note the urgent need for the latter activity in view of growing pressure to "develop" salt ponds in ways which would destroy indigenous hypersaline species.

Government-owned salt ponds ideally should be managed for long-term preservation, an activity which is for the most part compatible with <u>Artemia</u> culture. There is a potential conflict between use of the ponds for brine shrimp culture and their use as bird sanctuaries (many wading birds are quite fond of brine shrimp) which may require consideration.

b. Water Availability

Easy access to natural seawater is highly desirable for *Artemia* culture projects. This is seldom a problem, particularly in the case of ponds which have been used for salt production.

c. Water Quality

Eastern Caribbean *Artemia* are tolerant to a wide range of temperature, salinity, and oxygen conditions, and these factors should not be a problem if a provision can be made for periodic introduction of normal seawater. The possibility of organic pollution from domestic sewage and from pesticides used in local agriculture should be considered.

d. Liability to Weather Damage

Salt ponds are inherently susceptible to flooding by hurricanes or heavy rains. These events may destroy a current crop of brine shrimp, but lasting effects are not likely. The life cycle of these animals makes it possible to restore populations within a few weeks as long as a supply of cysts is maintained in safety.

e. Natural Supply of Juveniles

It is suggested that Eastern Caribbean culture operations be based on locally-occurring brine shrimp as opposed to imported strains. All of the islands surveyed had at least some ponds which contained brine shrimp, and it is important that the potential of these animals be established prior to modification of the gene pool with introduced strains. Adequate quantities of local innocula are available in all cases, and the oviparous mode of reproduction allows reserves to be maintained during periods of adverse climatic conditions.

f. Natural Supply of Feed

The existence of endemic brine shrimp indicates the presence of some forms of natural feed. The relationship of these to nutritional value of endemic *Artemia* and natural carrying capacity requires individual evaluation in each case. As noted above, where additional feed is needed to increase brine shrimp production, it is desirable to concentrate initially on local feed species.

3. Technical Capability

Simple harvesting of endemic brine shrimp requires no technical expertise. As noted above, estimated *Artemia* biomass in experimental ponds did not approach levels considered adequate for low-level production facilities elsewhere. The present study suggests two manipulations which might be applied to increase or sustain *Artemia* production: (1) Salinity control may be used to offset the effects of evaporation during dry seasons, and possibly to induce cyst production when desired. (2) Fertilization may be used to promote the growth of food organisms to increase carrying capacity or improve nutritional characteristics. Both manipulations can be accomplished inexpensively and do not require elaborate technology.

It must be emphasized, though, that these manipulations may not be effective in all cases, and may even be counter-productive (e.g., fertilization may promote the growth of undesirable species instead of those intended). The variability found between experimental ponds in the present study underscores the fact that it is unrealistic to suppose that specific pond management practices are generally transferable. Optimum (or acceptable) culture conditions must be developed empirically on each site.

Stabilization or enhancement of natural production requires ability to plan and execute comparative investigations, and may involve water quality, algal culture, or biochemical procedures which are not generally available in the Eastern Caribbean. Competent technical personnel are absolutely essential to this sort of operation. Similar technical capability may be needed if disease becomes a problem. Such expertise could be developed within the region through pilot projects, and extended through programs analogous to various agricultural extension programs which are currently operative in the Eastern Caribbean.

4. Legal

a. Exclusive Rights to Land/Water

Coastal lands are frequently considered to be public property, though this concept does not appear to extend to salt ponds. Exclusive access to seawater is highly unlikely, and any access will probably be contingent upon maintenance of natural water quality. Explicit government guarantees should be secured for access to pond, peripheral land, and seawater.

b. Control Requirements

At present, there are no restrictions which pertain to salt pond aquaculture in the Eastern Caribbean. This circumstance will very probably change in the near future through harmonized fisheries legislation and efforts to maintain the quality of coastal environments. Particular attention should be paid to the use of waste products as fertilizers (especially if there is outflow to the sea) and to any drugs which may be used for disease control. Restrictions pertaining to these activities are not likely to pose serious problems to Artemia culture. Drainage of salt ponds is sometimes rationalized as a means of eliminating mosquito breeding places or the source of objectionable odors. Brine shrimp culture offers an alternative to destruction of the salt pond. Maintenance of high salinity for brine shrimp culture will effectively eliminate mosquito larvae. Conditions which produce objectionable odors (usually sewage input) would probably be incompatible with Artemia culture, and hence would be eliminated as part of a culture project.

c. Insurance, credit availability, government incentives and political stability require site-specific evaluation. In general, insurance will be limited to personal insurance for employees. Credit is theoretically available through development banks, particularly if there is strong local involvement. In practice, credit may be difficult to obtain before pilot operations establish the economic viability of the mariculture venture. Most governments offer tax holidays and duty-free import concessions for pioneer industries. The outlook for political stability has improved substantially in recent months, but the region is still in a state of flux as newly independent island nations are evolving individual and collective styles of operation. It should be pointed out that, regardless of political stance, Eastern Caribbean governments usually approve of projects which are based on local resources and which generate local employment.

5. Manpower

Full development of Artemia culture in Eastern Caribbean salt ponds requires the following personnel categories: professional aquaculturist, technicians specialized in practical techniques, individual "farmers", extension agents, scientist specialists, processing specialists, and marketing specialists. It is not practical for a single brine shrimp culture operation to maintain this sort of personnel roster; this must be the responsbility of a regional institution, and almost certainly requires support from an international development assistance agency. In practice, specialists will probably not be needed on a continuing basis, and could be recruited on a consultant basis. More critical are extension agents and training programs to produce competent individual culturists to oversee daily operations. Managerial skill is notoriously lacking in small-island fisheries operations, and deserves attention from the outset in development efforts. There is often overriding interest in technical aspects of mariculture, but without dedicated management such efforts are almost certainly destined to fail.

6. Infrastructure

The elements of infrastructure essential to mariculture operations (research/training institutions, extension services, credit, transportation, storage facilities, processing facilities, marketing and distribution systems, cooperatives, communications, and product development) exist within the Eastern Caribbean as a whole, but are not presently coordinated or dedicated toward any project effort. Efforts to develop brine shrimp culture projects should place particular emphasis upon cooperative support from institutions and individuals currently active within the region.

In sum it is suggested that *Artemia* culture in Eastern Caribbean salt ponds can be viable, particularly as a cottage industry. The use of local waste products as fertilizers offers significant potential, and appropriate fertilization regimes need to be defined. Once site-specific methodologies have been established at a pilot scale, production may be maintained at a low-investment/low-technology level, or may be expanded with additional capital input. Pilot projects are strongly recommended to permit site-specific evaluation of potential pond manipulations.

C. Recommended Follow-Up

The need to conserve the Eastern Caribbean *Artemia* gene pool cannot be overemphasized. Salt ponds are widely perceived as having little importance, and the regional trend to develop tourist facilities can be expected to place brine shrimp habitats in growing jeopardy. This study has shown that Eastern Caribbean *Artemia* are genetically unique, are tolerant to more extreme environmental conditions than reference strains, and can have nutritional value equal to that of commercially marketed products. If there is no other sequel to this project, a comprehensive collection should be made of cysts from all productive ponds in the region and suitably prepared for archiving at the Artemia Reference Center in Ghent.

One or more pilot projects should be established with the intention of developing a commercially viable operation. While the economic analysis discussed above indicates that such projects are possible, locally specific values must be obtained for production and marketing parameters before the best development strategy can be identified. It is therefore suggested that the low-technology model be implemented, with a temporary applied research component to identify optimum salinity, fertilization, and harvesting regimes for the production of cysts and live biomass with fatty acid compositions which will make these products competitive with those produced elsewhere. The following components should be included in the optimization program:

1. Endemic algae should be screened to identify, isolate, and culture strains which have favorable fatty acid profiles and which thrive in hypersaline media. Maintenance of ponds at higher salinity levels can reduce or eliminate other animals (e.g., rotifers) which compete with _Artemia_ for food under other conditions. Particular attention should be paid to identifying fertilization schemes which favor the production of small cells with high carbohydrate content and larger cells with high protein and lipid components, as this combination has been shown to provide a particularly suitable diet for _Artemia_ (Johnson, 1980).

2. Experimental fertilization (preferably with locally available waste products) should be conducted in salt ponds intended for _Artemia_ culture, so that the minimum level of eutrophication needed to optimize _Artemia_ production can be determined.

3. Certain ponds might be raked initially to improve suspension of benthic food and/or nutrients. These should be quantified (as total carbon, or better, carbon, lipid, carbohydrate and protein). _Artemia_ production in ponds treated in this way should be compared with those receiving fertilizers.

4. An initial salinity of 90 o/oo is suggested as a starting point for optimum production. This may have to be increased to induce cyst production along the lines suggested in this report. Isolation of segments of salt

ponds with levees can be advantageous in reducing the effects of surrounding watersheds on pond salinity.

It is critical that the development of pilot projects for commercial production of *Artemia* in the Eastern Caribbean be cognizant of the overall development objectives of the host country. The need to generate employment may have to be balanced against the desire to generate foreign exchange, and other operative projects in specific countries may affect the local view of what types of development are needed. The lower-level project proposed as follow-up is advantageous in that there is greater potential for local investment (and ultimately local control) than would be the case in the alternative scenario.

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NOTE: In the following citations, "The Brine Shrimp _Artemia_" refers to Persoone, G., P. Sorgeloos, O. Roels, and E. Jaspers, (editors), 1980. The Brine Shrimp _Artemia_. Universa Press, Wetteren, Belgium.

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		Title Page	one side
page	i	Contents Page	one side
pages	ii-iii	List of Tables	back to back
	iv-v	List of Figures	back to back
	vi-vii	Acknowledgements and p. vii	back to back
	viii	end of Executive Summary	one side
	1-26	--	back to back
	27		one side
	28-111		back to back
	112		one side
	113-116		back to back
	117		one side

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