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Monterey, California

THESIS

THE USE OF COMPUTER INTENSIVE STATISTICAL MODELING IN ESTIMATING THE VARIABILITY OF MARINE FOULING COMMUNITIES

by

David L. Martin

June 1983

Thesis Advisor: E. C. Haderlie

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were then used to determine an appropriate strategy for sampling the fouling community in Monterey Bay. The results indicate that twenty to thirty plates are required to resolve ambiguities concerning the mean percent cover of a group of plates while many more are required to quantify the variability of the fouling population.

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The Use of Computer Intensive Statistical Modeling in Estimating the Variability of Marine Fouling Communities

by

David L. Martin
Lieutenant, United States Navy
B.S., University of Washington, 1976

Submitted in partial fulfillment of the
requirements for the degree of

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ABSTRACT

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I. INTRODUCTION

A. GENERAL

The term biofouling refers to the settlement, attachment, and growth of marine organisms on surfaces that man puts into the ocean. This process has a profound impact on naval operations due to the fouling of ships hulls, rudders, salt water piping systems, sonar domes, and the fouling and biodeterioration of harbor or pier structures. This problem has been estimated to cost the Navy several hundred million dollars a year (Woods Hole,1952; Fisher et al,1975) due in part to increased fuel requirements caused by the greater frictional drag of fouled ships hulls, the increased repair or replacement cost of piping and machinery damaged by fouling organisms, and the need to expend funds to continually remove the fouling organisms from vessels.

The principal method used to combat the problem of biofouling on the hulls of ships has been the application of antifouling paints. In general, such paints contain any of several metallic compounds which, as they leach out of the paint matrix, are toxic to fouling organisms.

The testing of antifouling paints prior to their release for general use involves the use of sampling assay techniques. In simple terms, a number of substrates are

painted with the paint to be tested and are deployed in the sea for some arbitrary length of time. Following the retrieval of these substrates, they are compared with non-toxic control surfaces that have been exposed for the same length of time and the efficacy of the antifouling paint in preventing the settlement of fouling organisms is ascertained.

1. Sampling Design

The proper number of plates to deploy for the testing of antifouling paints, or for that matter, the study of fouling organisms in general, has always been somewhat arbitrary. This is because the proper number of plates to deploy to sample the fouling population is a direct function of the degree of variability within the population. Despite the great volume of fouling research that has been conducted, the study and quantification of this variability has only very recently been attempted.

2. Previous Research on Fouling Community Variability

Most of the information dealing with the variability of biofouling communities has been collected within the past decade and is often somewhat contradictory. This contradiction is sometimes caused by the particular descriptors of the fouling community (percent cover, species counts, etc.) the researchers used in the analysis of its variability.

Research concerning the variability of small numbers of panels suspended for only one month off the Florida coast (Mook,1976) showed very little variability in species count. These results should be interpreted cautiously however due to the short time of immersion.

Similar research conducted in North Carolina (Sutherland,1974) using a more extensive series of panels suggested that the development of the fouling community was extremely variable. This research supported the conclusion of earlier studies in California (Boyd,1972) which also found significant fouling community variability.

Studies conducted in Hawaii (Schoener et al,1978) and in Massachusetts (Osman,1977) found that fouling community variability based on species counts was relatively low. This conclusion was echoed by a study which analyzed the variability of identical panels in terms of total percent cover, species count, and inter-panel similarity indices (Schoener and Greene,1980). The results of the study indicated that approximately ten replicate panels were sufficient to resolve to a high degree of confidence, the mean value of these descriptors.

As is evident, there is wide disparity between the various studies conducted to date on what the variability of the biofouling community at various locations truly is. Before meaningful antifouling paint test procedures can be

developed, proper sampling techniques based on the quantification of fouling community variability must be devised.

B. OBJECTIVE

The primary objective of this thesis was to determine the variability of the biofouling community in Monterey Bay. Once this had been completed, the development of a appropriate sampling strategy based on this variability could be accomplished. This information was to be provided to the David Taylor Naval Ship Research and Development Center so that modifications to present antifouling test procedures could be undertaken as required.

II. METHODS AND MATERIALS

A. GENERAL

The experiment consisted of deploying one-hundred (100) mild steel plates, each painted with one of four vinyl or epoxy based paints, in Monterey Harbor (Figure 1) for periods of up to 11 months and determining the variability of the resulting fouling communities that settled. Three of the paints used in the study contained antifouling compounds while the fourth (the control surface) did not. The fouling community structure on each plate was determined destructively (the plates were not redeployed after study) by microscopic analysis and the fouling population and makeup were determined.

B. PLATES AND PAINTS

The one-hundred plates used in this study were fabricated from low carbon, mild (structural) steel with dimensions 25.4cm x 30.5cm x .16cm . A small (.64cm) hole was drilled approximately 1.3 cm down from the midpoint of the top edge (one of the edges with the lesser dimension) of the plates to allow for the attachment of the suspending line for deployment. Additionally, a small (.32cm x 2cm) groove was milled through the plate approximately 6.5cm to one side of the drilled hole for the attachment of the

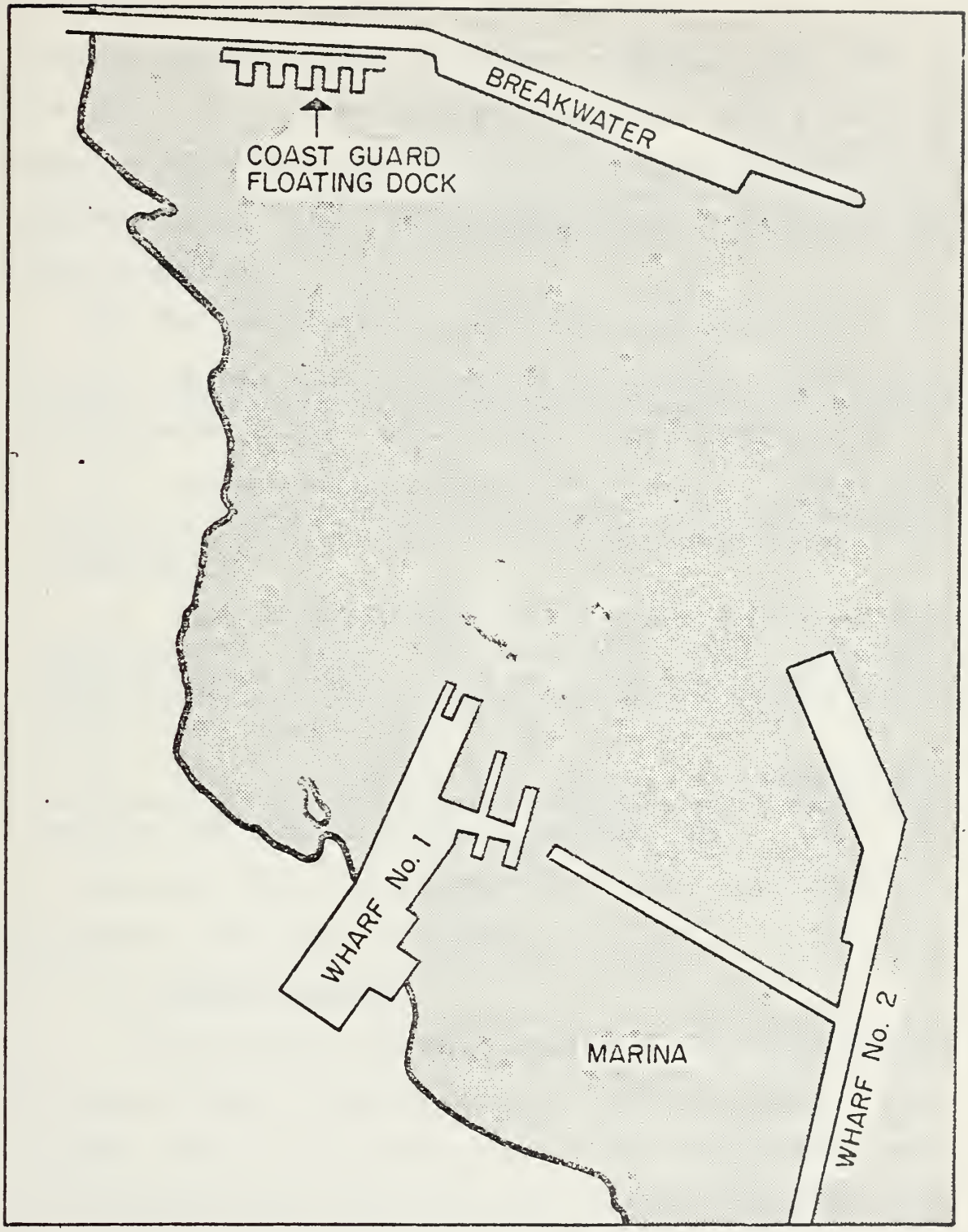

Figure 1. A Diagram of Monterey Bay Showing the Deployment Site at the Coast Guard Floating Dock.

identification tag. For consistent reference, the front of the plate was chosen as that side which would face the observer when the plate was held vertically with the drilled hole at the top and the identification groove to its' left (Figure 2).

The four paints used in the experiment were:

1. MICRON 22; a commercially available antifouling paint containing bis(tributyltin) oxide and cuprous thiocyanate as the antifouling agents (Appendix A).

2. Navy Standard Formula 121 Red Vinyl Antifouling Paint; the discontinued U.S. Navy antifouling paint containing cuprous oxide as the antifouling agent (Appendix B).

3. Navy Standard Formula 170 Black Camoflage Vinyl Antifouling Paint; the currently used standard antifouling paint of the U.S. Navy containing bis(tributytin) oxide and tributyltin fluoride as the antifouling agents (Appendix C).

4. Zynolyte Epoxy Rust Mate; a commercially available non-toxic corrosion resistant epoxy based paint used as the control surface (Appendix D).

Allplates were sandblasted then primed and painted in accordance with label directions.

1. Priming Procedure

The priming procedure consisted of first applying one coat of Navy Standard Formula 117 'Green Wash Primer' to both sides of all plates with a 5cm latex rubber brush and allowing this to dry for twenty-four hours. This was followed by the application of two coats of Navy Standard Formula 119 'Red Lead' to both sides of all plates using a 7.6cm nylon paint roller. The first coat of Formula 119 was

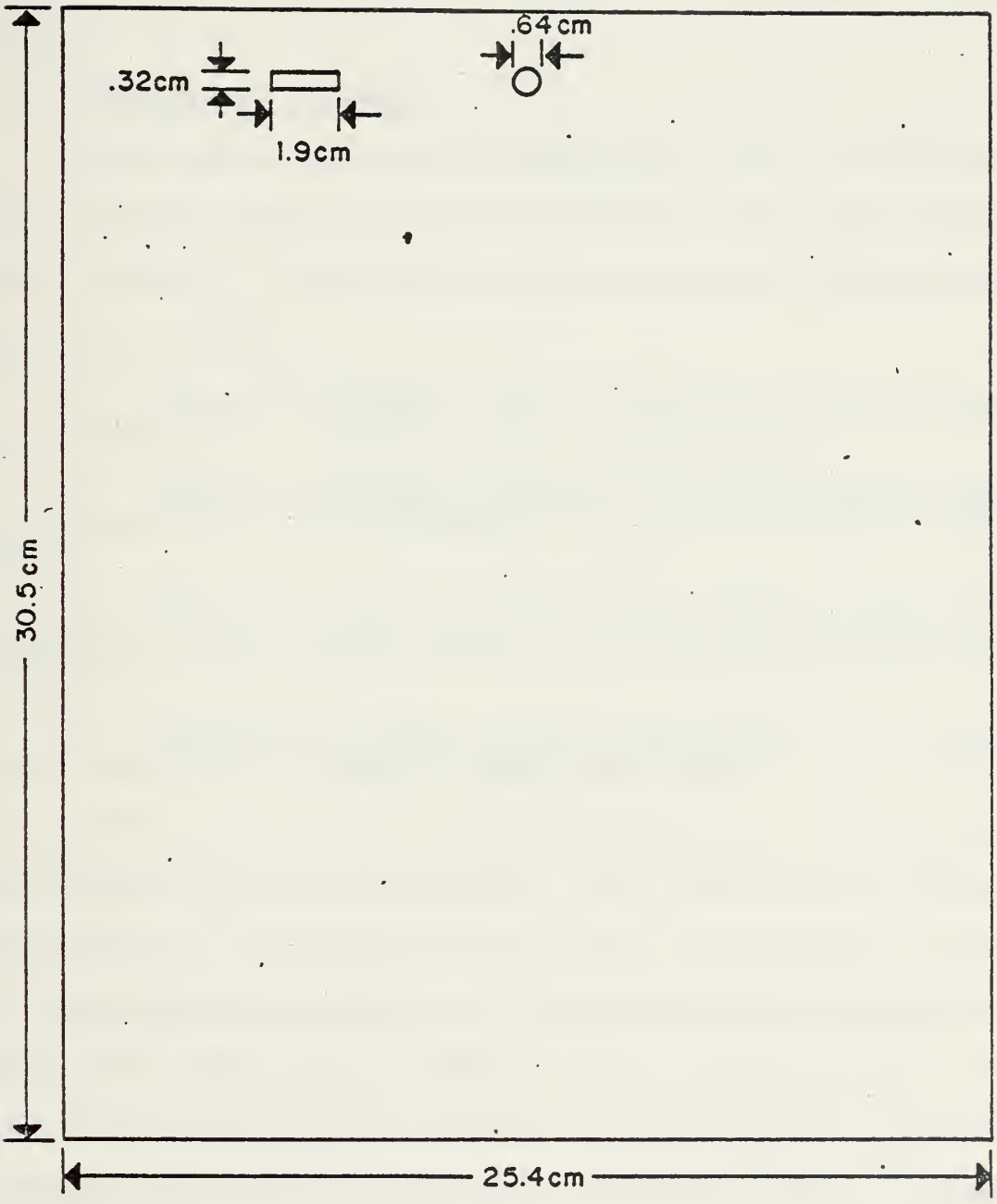

Figure 2. The Front Side of One of the Experimental Plates.

allowed to dry for forty-eight hours before the second coat was applied, and an additional forty-eight hours drying time was allowed prior to painting with the vinyl or epoxy paints.

2. Painting Procedure

All painting was accomplished using 7.6cm nylon paint rollers. The plates were divided into four groups (A,B,C, and D) of twenty-five plates each and labelled and painted as follows:

1. Plates A1 through A25 were painted on both sides with one coat of MICRON 22 antifouling paint.

2. Plates B1 through B25 were painted on both sides with one coat of Navy Standard Formula 121 Antifouling Paint.

3. Plates C1 through C25 were painted on both sides with one coat of Navy Standard Formula 170 Antifouling paint.

4. Plates D1 through D25 were painted on both sides with one coat of Zynolyte Epoxy Rust Mate.

The paint on all plates was then allowed to cure for 96 hours prior to deployment. The plates were labelled sequentially in each group (A1,A2,etc) by affixing, through the identification groove, an embossed DYMO tape label to each plate using monofilament nylon fishing line. This method of labelling was chosen to prevent the catalytic corrosion problems attendant with standard bronze or copper tags in contact with the steel plates.

C. DEPLOYMENT PROCEDURE

The plates were randomly divided into twelve groups of eight plates consisting of two plates from each of the four paint groups. To each plate a one meter length of .32 cm diameter stainless steel cable was then affixed by passing the cable through the drilled hole and forming a loop which was closed using Nico-Press crimp fittings (Figure 3). A similar loop was formed at the distal end of the cable to facilitate attachment at the deployment site.

On 22 and 23 May 1982, the plates were suspended (by groups) beneath the service access covers that extend the length of the floating dock at the Coast Guard Station Monterey. Each plate was individually deployed by attaching the distal loop of the cable to 10d nails driven into the dock and allowing the plate to hang vertically in the water (Figure 4). The plates were separated by a minimum horizontal distance of 40cm with the tops of the plates approximately one-half meter beneath the surface of the water. Since the dock rose and fell with the tide, the depth of immersion remained constant. Water depths below the plates (at MLLW) ranged from approximately three meters depth at the shallower end of the dock to more than ten meters depth at the seaward end. After being submerged for one month, inspection of the plates revealed that significant galvanic corrosion had occured at the junction

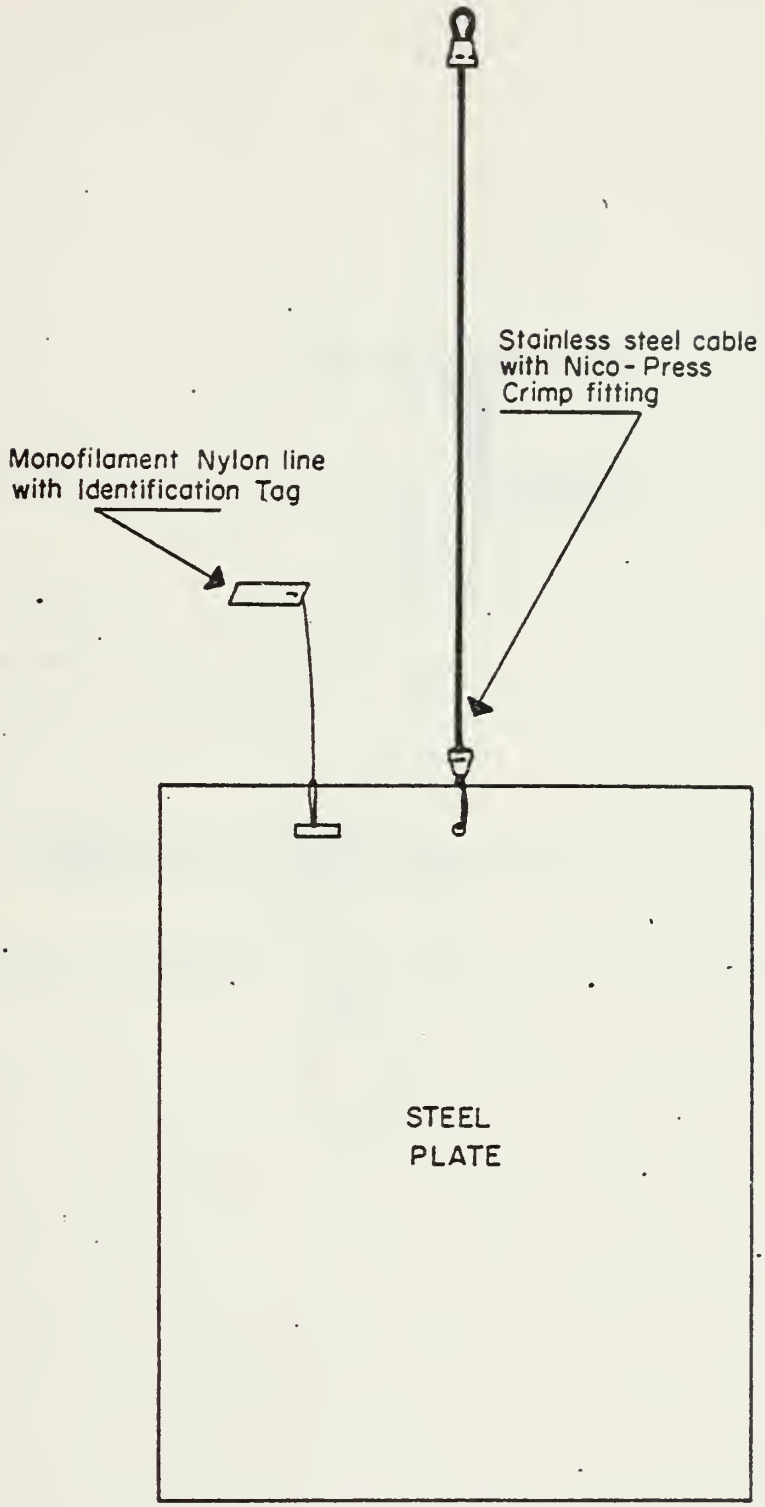

Figure 3. A Drawing Showing the Method of Attachment of the Suspending Cable and Identification Tag.

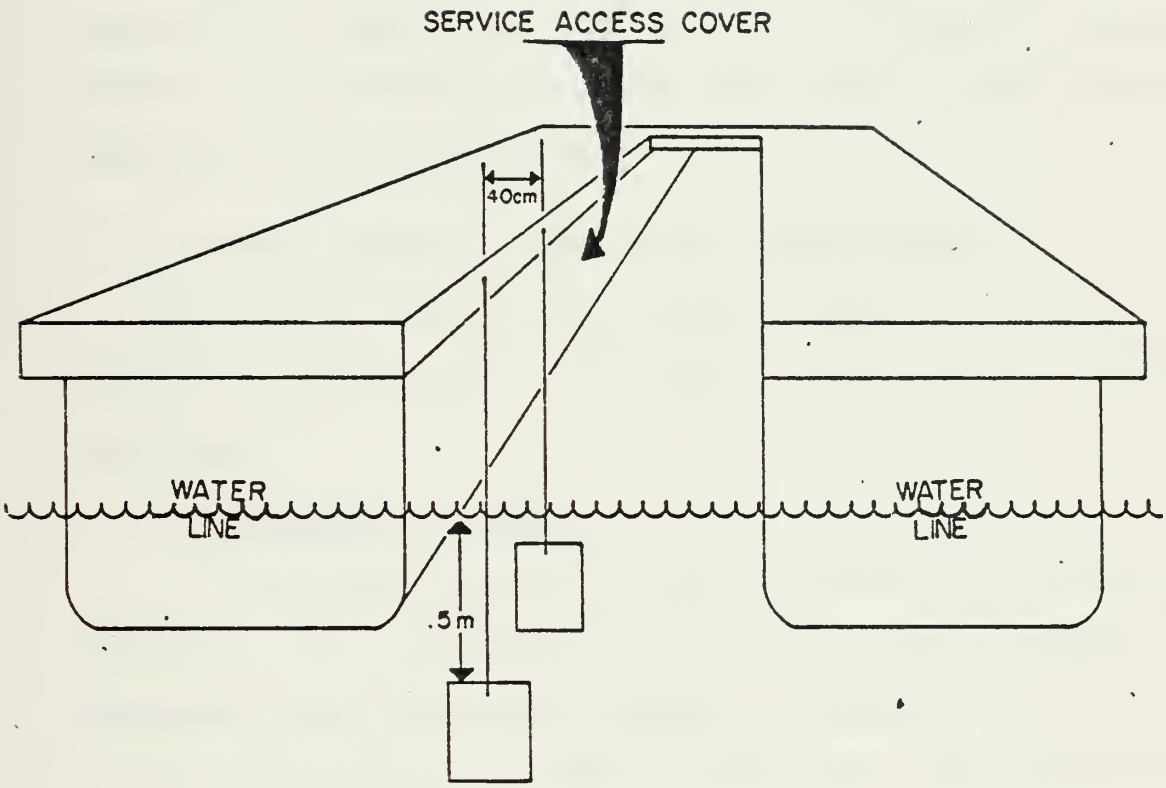

Figure 4. A Perspective View in Cross Section Showing the Deployment of the Plates at the Coast Guard Dock.

between the mild steel plates and the stainless steel cable. As a result, on 23 June 1982, all stainless steel cables were removed and replaced with .95 cm diameter nylon line. This necessitated the exposure of each plate to the atmosphere for approximately thirty seconds during the replacement operation but , since the plates remained moist, it was felt no harm was done to the fouling organisms that had settled.

D. FOULING COMMUNITY CENSUS AND IDENTIFICATION

Each month following the initial deployment, one of the groups of eight plates was randomly selected for retrieval and study.

1. Sampling Procedure

For each group of plates, a sampling grid of one-hundred (100) uniformly distributed random points was generated and graphically plotted by computer on a 25.4 cm x 30.5 cm output sheet. These points were then transferred manually to a clear plexiglass cover. The fouling communities on the plates were then systematically analyzed by setting the plates horizontally in a shallow container filled with seawater and positioning the plexiglass cover over the top coincident with the edges of the plate. Animals beneath plotted points were then censused and identified through the use of a stereo microscope. Since the plates had been suspended vertically in the water, none

of the sedimentation problems associated with horizontal deployment strategies developed. Therefore both sides of the plates were analyzed and counted as separate substrates.

2. Identification

To eliminate the necessity for compound microscope identification of settled organisms and to negate the effects of neuston contamination of census results, only sessile, attached organisms greater than .5mm in size were counted and identified. Identification was accomplished through the use of available keys and literature; (Osburn,1952; Knight Jones,1979; Haderlie,1974; Morris et al,1980; Frazier,1937; Smith and Carlton,1975). In all, some thirty-two taxa were identified as major space occupiers during the study (see Appendix H for species list).

III. STATISTICS

A. EXPERIMENTAL DATA

The data obtained from the census and identification of the organisms on the plates retrieved each month was used to generate the statistics that described the fouling populations. The two main descriptive quantities used to assess the variability of the fouling community were the total percent fouling cover on each plate and the similarity of the fouling organizations between plates.

1. Percent Cover

The initial estimate of the total percent fouling cover on each plate was calculated by dividing the number of points from the census that had organisms beneath them (this value was termed S_t) by the total number of points censused per plate (N_t = 100 for all plates). Since this method was obviously subject to some unknown degree of uncertainty, revised estimates for the total percent fouling cover were made using statistical techniques described below.

2. Similarity

The similarity between the fouling communities on the plates retreived each month was determined by the calculation of the Bray-Curtis similarity index, I_a (Whitaker,1952). This index is defined as:

$$I_a = \sum \min(a,b)$$

where a and b are the fractional species proportions present on plates A and B. These fractional species proportions were determined from the initial census data by dividing the total number of instances a particular species was counted during the plate census by 100. In this study, empty points not occupied by any organism were treated as a separate species. As an example, suppose the following data were collected:

Species	Proportion on Plate A (=a)	Proportion on Plate B (=b)	min(a,b)
Empty	.30	.24	.24
Species #1	0	.11	0
Species #2	.46	.42	.42
Species #3	.24	.23	.23

then the Similarity Index = $I_a = \sum \min(a,b) = .89$

The similarity index is thus a measure of the degree of variability between the fouling communities on the two plates in terms of both the total percent cover and the species composition.

The main purpose of calculating the similarity index between plates was to examine whether any of the antifouling coated plates displayed more variability than did the non-toxic control surfaces. This was of particular interest since a previous thesis (Kelley,1981) which dealt with marine microfoulers in Monterey Bay had shown that surfaces coated with an organo-metallic antifouling paint

served as attractants to the organisms and, hence, were more heavily fouled, showed greater species diversity , and were more variable in terms of their fouling communities than were the control surfaces. Provided that the macrofouling community investigated in this thesis did not behave similarly, that is if the fouling communities on the non-toxic control surfaces were consistently more variable than were those on the antifouling coated surfaces, then any sampling strategy which could discern to an acceptable degree of error the amount of variability of the communities on the control surfaces would be able to do at least as well concerning the antifouling coated surfaces. The thrust of this thesis was to first investigate whether or not the control surfaces for each month exhibited more variability than did the antifouling coated surfaces. Provided this criteria was met, the next step was to devise an appropriate sampling strategy for the control surfaces using sophisticated statistical modelling to extrapolate the data obtained from the four control surfaces retrieved each month to any desired number of simulated samples.

B. STATISTICAL MODELLING

Once the initial percent fouling coverage estimates for the four non-toxic control surfaces retreived each month were determined, the data had to be manipulated to obtain better estimates of the variability of the superpopulation

of marine fouling organisms the plates were assumed to be sampling. Since there were only four substrates examined per month, a model capable of extending the experimental data to simulated samples of any size was required. The model also had to be capable of allowing and quantifying inter-plate variability within the simulated sample set. The techniques used to successfully meet these requirements have only recently been developed and this thesis is apparently the first incorporation of these techniques into fouling research.

1. Model Alternatives

One of the methods that has been used in the past (Schoener and Greene, 1980) to examine the degree of variability of biofouling coverage values has been to assume that the fractional coverage estimates have a normal distribution. Using this simple model, the upper and lower 95% confidence limits are calculated about the experimental mean percent fouling cover by use of the formula:

$$\text{the 95\% Confidence Limits} = \bar{x} \pm 1.96 * \hat{\sigma} / \sqrt{N}$$

where $\bar{x}$ is the mean percent fouling cover, $\hat{\sigma}$ is the experimental standard deviation, N is the number of samples examined , and the formula is derived from the standard normal distribution. By assuming that the mean ($\bar{x}$) and standard deviation ($\hat{\sigma}$) do not vary with the number of plates examined, one varies N in the formula to determine

the effect that the number of plates has on the approach of the upper and lower confidence limits to the experimental mean. It must be pointed out however that this model has serious drawbacks. The first problem with this approach is the assumption that fractional fouling coverage values which will always lie between zero and one can be described by a normal distribution that is unbounded in range. While this assumption permits the calculation of various statistical parameters using well known analytical formulas, it is obviously weak theoretically. Secondly, this model requires that the mean and standard deviation are stable with increasing sample size. This requirement has the effect of forbidding inter-plate variability. Not only is such a restriction biologically untenable, it reduces the formula for the calculation of the confidence limits to :

$$\text{the 95\% Confidence Limits} = [+/-]\ \text{constant}/\sqrt{N}$$

Note that the above equation will result in a curve similar to $[+/-]\ 1/\sqrt{N}$ when plotted. Since the value of $1/\sqrt{N}$ decreases by nearly 70% as N goes from one to ten, use of this method will always show that approximately ten plates are sufficient to resolve the variability of the mean percent cover. This result however is merely an artifact of the simplistic model used in its calculation.

Another possible method that could be used to estimate the degree of variability of the biofouling

community would be to invent a predictive model for fouling populations. Unfortunately, such a model is considerably beyond our abilities at the present time. Such a model would require advective/diffusive models accurate spatially to microscale resolution . It would also require the ability to parameterize the entire range of biological forcing functions which include predation, nutrient supply, susceptibility to environmental fluctuations, behaviorism of the organisms involved and planktonic larva survival rates to name just a few. Obviously, such sophistication in a model is not likely in the forseeable future.

Since a stochastic model which assumed the fouling coverage values had a normal distribution was insufficient due to its' inherent restrictions which forbid interplate variability, and since a predictive model was unattainable, this study used a probabilistic model to explore the variability of the fouling community. In such a model, the variability of the initial experimental data is determined and the statistical descriptors of that varibility are used as inputs for computer simulations of the experiment.

2. Procedure

Each plate (t) was assumed to have a random,independent proportion of fouling, P_t. P_t was assumed to be of the form:

$$P_t = \frac{e^{\varepsilon_t}}{(1 + e^{\varepsilon_t})}$$

where ϵ_t was assumed to be normally distributed with unknown mean (μ) and variance (σ^2) which were independent of the other plates censused at the same time. Note that the above equation could be written as:

$$\epsilon_t = \ln(P_t / (1 - P_t))$$

Each month, the ϵ_t value for each of the four untreated plates was determined as were the mean and variance of the four ϵ_t values. Revised estimates for these parameters were then determined using the Method of Maximum Likelihood (see Appendix E for mathematical development).

Using as inputs the maximum likelihood values for the mean ($\hat{\mu}$) and variance ($\hat{\sigma}^2$) of the monthly epsilon values, bootstrap computer simulations (Efron,1979) of the experiment were conducted. The bootstrap method was used to simulate 200 groups of various fixed numbers of plates (see Appendix F for a complete discussion).

Using the results from the bootstrap computer simulations, the following statistics were calculated:

1. The expected value of the mean percent fouling coverage. This was determined by calculating the mean of the 200 simulated group means for each of the various numbers of plates per group simulated.

2. The upper 95% confidence limit about the expected value of the mean percent fouling cover for each of the various numbers of plates per group simulated. This was estimated by ordering the mean percent foulng coverage values for each of the 200 simulated groups of plates in ascending order and then finding the 95% quantile of the expected mean percent fouling coverage values.

3. The percent fouling cover that one can say 90% of the simulated plates will have fouling coverage less than or equal to (with 95% confidence). This was done by finding the 95% quantile (as described above) of the parameter NEWCVR(I) where:

$$\text{NEWCVR(I)} = e^{\beta}/(1 + e^{\beta})$$

$$\text{and } \beta = \hat{\mu}(\text{I}) + 1.285 * \hat{\sigma}(\text{I})$$

Note that $\hat{\mu}(\text{I})$ and $\hat{\sigma}(\text{I})$ are the bootstrap simulations of the mean and standard deviation of for each of the 200 group simulations. The value 1.285 is the 90% quantile for the standard normal distribution.

The purpose of calculating the parameter NEWCVR was to estimate how many plates it would take to be 95% confident of capturing 90% of the variance of the biofouling population. The confidence and variance values chosen were arbitrary, and the model can be readily modified to estimate the number of plates required to ascertain any degree of variance to any desired confidence.

4. The standard deviation about the mean percent fouling coverage that 90% of the simulated plates would have was also estimated . This was done by finding the mean and standard deviation of the 200 NEWCVR(I) values for each of the various numbers of plates per group simulated each month.

IV. RESULTS

A. GENERAL

Experimental data were collected monthly and the initial percent cover and similarity values computed (and the coverage estimates tabulated) for the second through the eleventh months of the experiment (Appendix G). Following the procedure discussed in the statistics chapter, these initial estimates were used as starting values for Bootstrap/Maximum Likelihood computer simulations of the experiment. The computer simulation results from month 2 are described in some detail. The simulation using the data from months 3 through 11 followed the same procedure and are each presented in a brief synopsis.

1. Method Verification

The procedure described in this thesis to develop an appropriate sampling strategy for antifouling paint test purposes was based on the assumption that any strategy which could ascertain the variance of the biofouling community on non-toxic control surfaces would be able to do at least as well regarding antifouling coated surfaces. This assumption would be correct provided the fouling communities on non-toxic control surfaces were more variable than were those on the antifouling coated surfaces. By

analyzing the monthly mean similarity values for the fouling communities on these two types of surfaces (Figure 5), it is clear that the antifouling coated surfaces displayed consistently less variability in their fouling structure than did the control surfaces. Therefore, this assumption is considered to be quite strong.

A second major assumption used in the development of this procedure was that the monthly epsilon (ϵ_t) values used in the calculation of the proportion of fouling (P_t), were independent random values from a normal distribution with mean (μ) and variance (σ^2).

If the epsilon values were in fact distributed normally, then dividing the individual epsilon values for each month by the monthly standard deviation of the epsilon values and subtracting the mean monthly value of epsilon from this result, would transform the epsilon distribution into the standard normal distribution. By plotting the (N) transformed epsilon values ranked in ascending order against the theoretical order statistic obtained by finding the inverse function of the standard normal distribution for (j/N+1) as j goes from 1 to N, a diagnostic plot of the model was obtained. If the model was perfect, there would be a one-to-one correspondence between the transformed epsilon values and the order statistic. Plotting the forty transformed epsilon values obtained in this study against

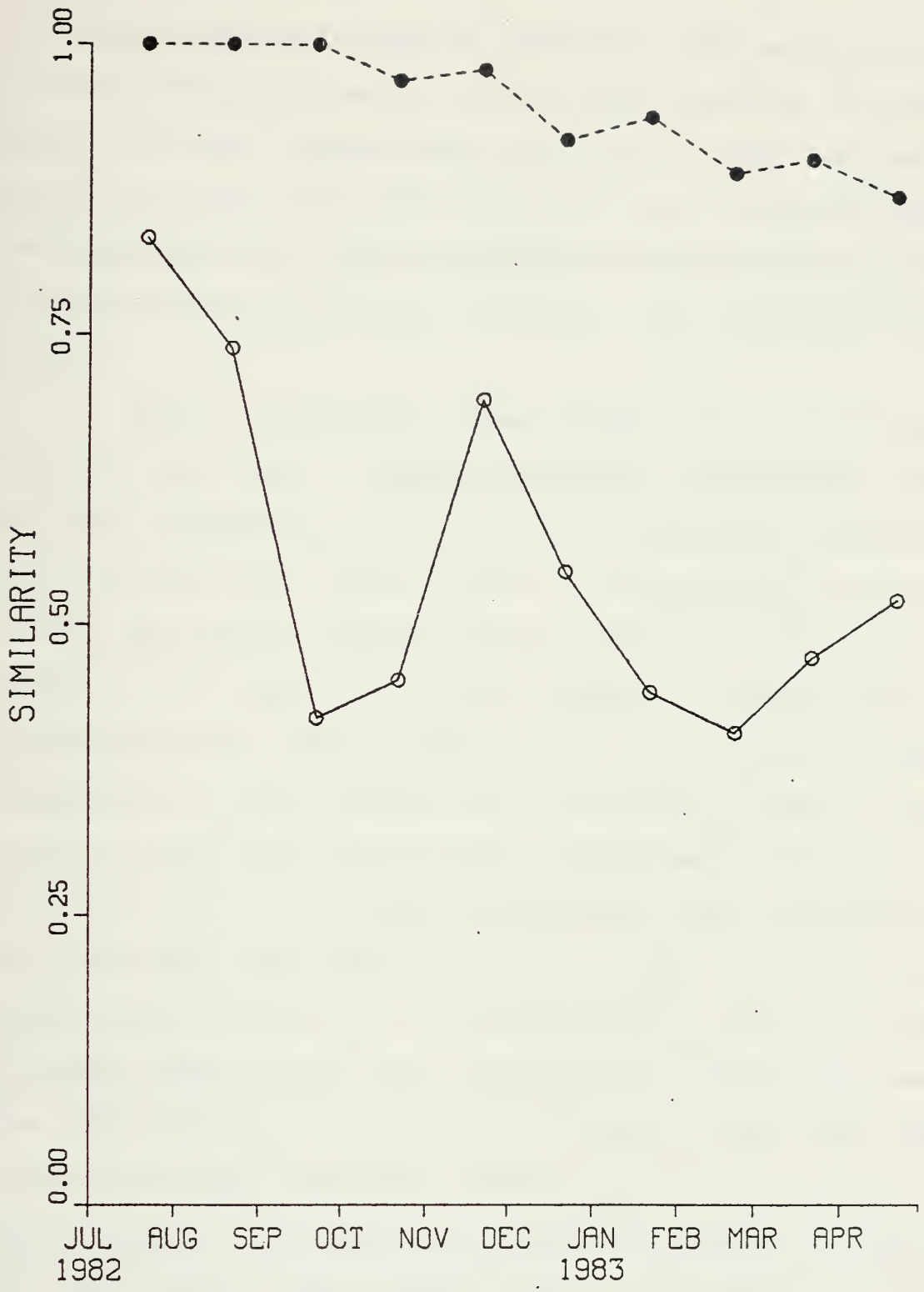

Figure 5. Chart Showing the Monthly Mean Similarity Values for the Control Surfaces (Solid) and the Antifouling Coated Surfaces (Dashed).

the order statistic (Figure 6) shows that the assumption of a normal distribution for the variable epsilon is quite good. The least squares best fit line for the data had a slope of 1.07 (vice 1.00 for the theoretical perfect correspondence) and the correlation coefficient between the transformed epsilon values and the order statistic was 0.98.

Since the initial experimental data for percent fouling cover and the Bootstrap/Maximum Likelihood model were not independent but rather were coupled by the estimates of the mean and variance of the monthly epsilon values, one would reasonably expect the final mean percent fouling cover predicted by the Bootstrap simulations to approximate the actual data value if the model behaved reasonably. A chart comparing the arithmetic mean of the monthly values for percent cover obtained experimentally to the expected value of the mean percent cover predicted by the bootstrap simulations (Figure 7), shows that the model agrees quite well with the experimental data. In those instances where there was a significant difference between the mean percent cover obtained from the data and that predicted by the bootstrap model, analysis of the actual data suggested that simply finding the arithmetic mean of the four monthly data percent coverage estimates was perhaps too sensitive to unusually high or low values.

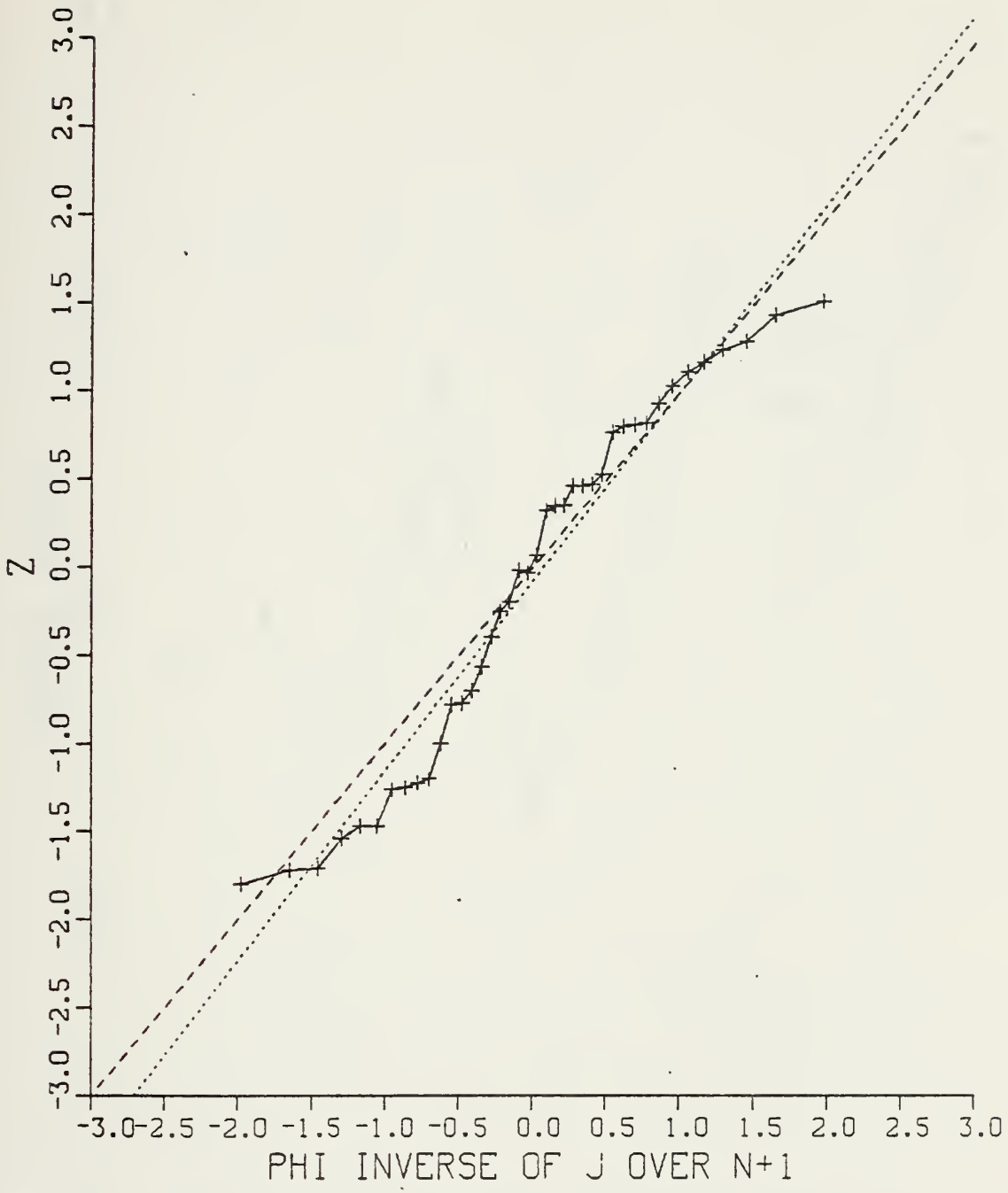

Figure 6. A Diagnostic Plot of the Model With the Ranked, Normalized Values for the Individual Epsilon Values Plotted on the Ordinate (Abbreviated as Z) and the Theoretical Order Statistic Plotted of the Abscissa. The Dashed Line Indicates Perfect Correspondence and the Dotted Line is the Least Squares Best Fit for the Data.

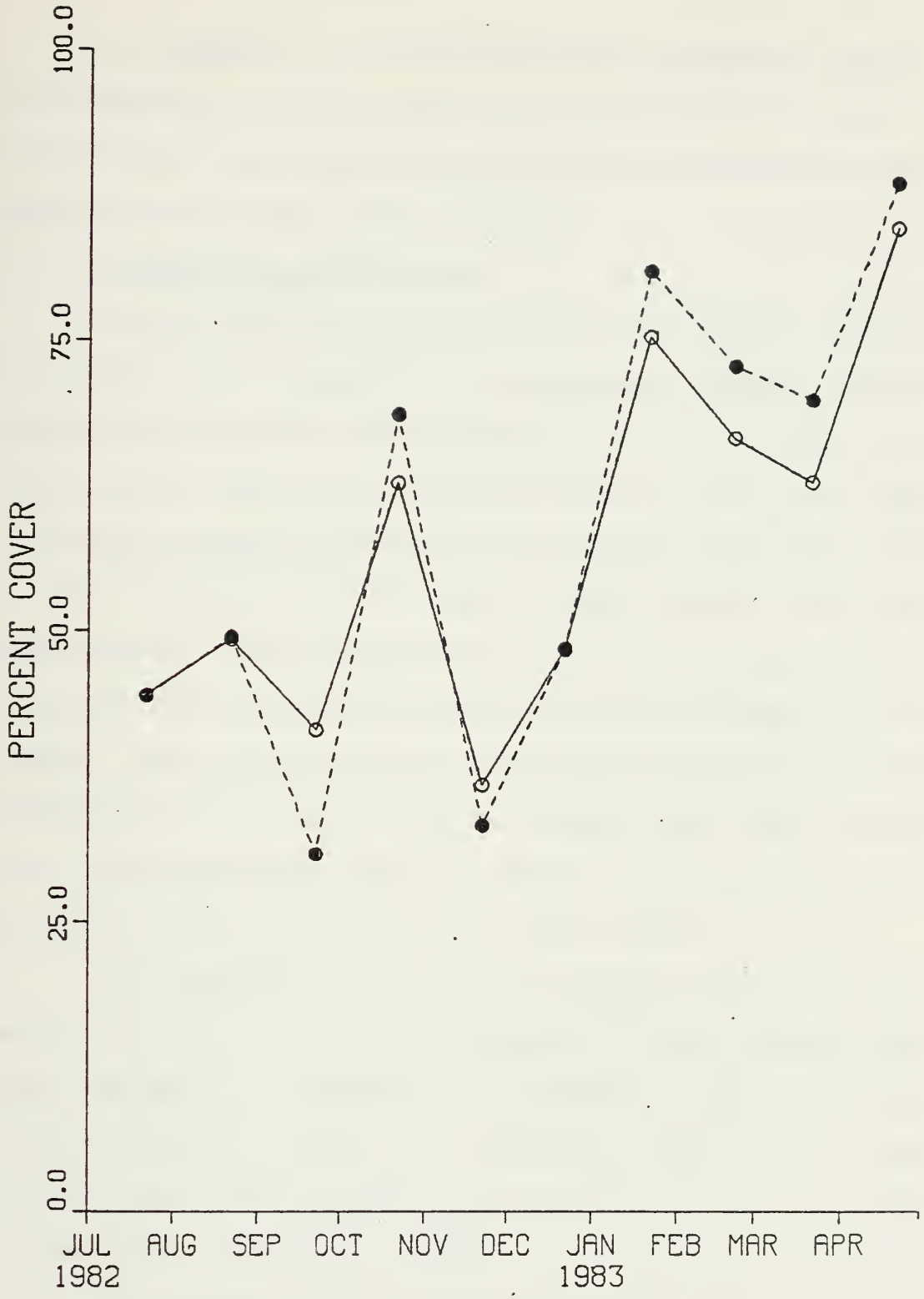

Figure 7. Chart Showing the Arithmetic Mean of the Percent Fouling Cover From the Experimental Data (Solid) and the Bootstrap Simulated Mean Percent Cover (Dashed).

In summary, it is felt that the assumptions made in the formulation of this model are quite good and that the model gives very reasonable estimates of the most likely value for the percent fouling cover.

2. Explanation of Figures

The similarity indices between the plates retrieved each month were plotted on two separate graphs for the control surfaces and the antifouling coated surfaces. The range of the similarity values from zero to one was plotted on the ordinate and the plate designations were listed on the abscissa. The 'F' and 'B' that follow the plate group number refer respectively to the front and back of the plate. To obtain the similarity value between any two plates, find the horizontal line above the abscissa with arrowheads which terminate at points above the desired plate designations, then proceed horizontally to the ordinate to find the similarity index value.

The expected value of the computer simulated mean percent fouling cover and the upper 95% confidence limit about the mean as functions of increasing sample size were plotted for each month. The expected value of the mean percent cover was dispayed as a solid line and the upper 95% confidence limit was dashed.

Graphs were also prepared showing the results of the monthly computer simulations used to determine the 95%

confidence limit of the percent fouling coverage that 90% of the simulated plates will have fouling coverage less than or equal to. The ordinate of these figures was graduated in percent cover from zero to one hundred percent. The abscissa was labelled with the number of plates per group used in the simulations. The 95% confidence limit for the percent cover of 90% of the plates was then plotted as a function of increasing sample size.

The same units for the ordinate and abscissa were used in the figures showing the standard deviation about the mean percent cover that 90% of the plates would have as functions of increasing sample size. For these figures, however, the percent cover label on the ordinate refers to the deviation about the mean percent cover and not the total percent fouling cover these plates will have.

B. RESULTS FROM MONTH 2

1. Experimental Data

The fouling community on the control surfaces after two monhs immersion was dominated by the hydroid Obelia spp. Ectoproct colonies each consisting of several dozen zooids had also settled by this time. These initial bryozoans were primarily Hippothoa hyalina and Celloporaria brunnea although the ancestrula stage of a recently introduced species, Watersipora cucullata was also present.Watersipora is indigenous to the Galapagos Islands

and has not been reported in the literature farther north than the Gulf of California (Osburn,1952). It has not been noted in fouling studies conducted in Monterey Bay over the last twenty years and its appearance on the plates in this study is probably due to the abnormally warm coastal waters caused by the recent El Nino event.

The similarity values for the control surfaces ranged from .74 to .92 with a mean of .83 (Figure 8A). The lack of any settlement on the antifouling coated surfaces resulted in similarity values of 1.0 for all of those plates (Figure 8B).

2. Computer Simulations

The bootstrap computer simulation of the expected mean percent fouling cover (Figure 9) resulted in a final iterated estimate of 44.4% for the simulated mean percent cover (vice 44.5% for the data arithmetic mean). Note that the dashed line indicating the upper 95% confidence limit of the mean percent cover does show some inverse relationship to N (the number of samples) but does not show the precipitous approach to the mean as N goes from one to ten that was predicted by the model which assumed a normal distribution for the fractional coverage values.

Perhaps the most striking result of the computer simulations was that of the effect of increasing the number of simulated plates sampled on the 95% confidence

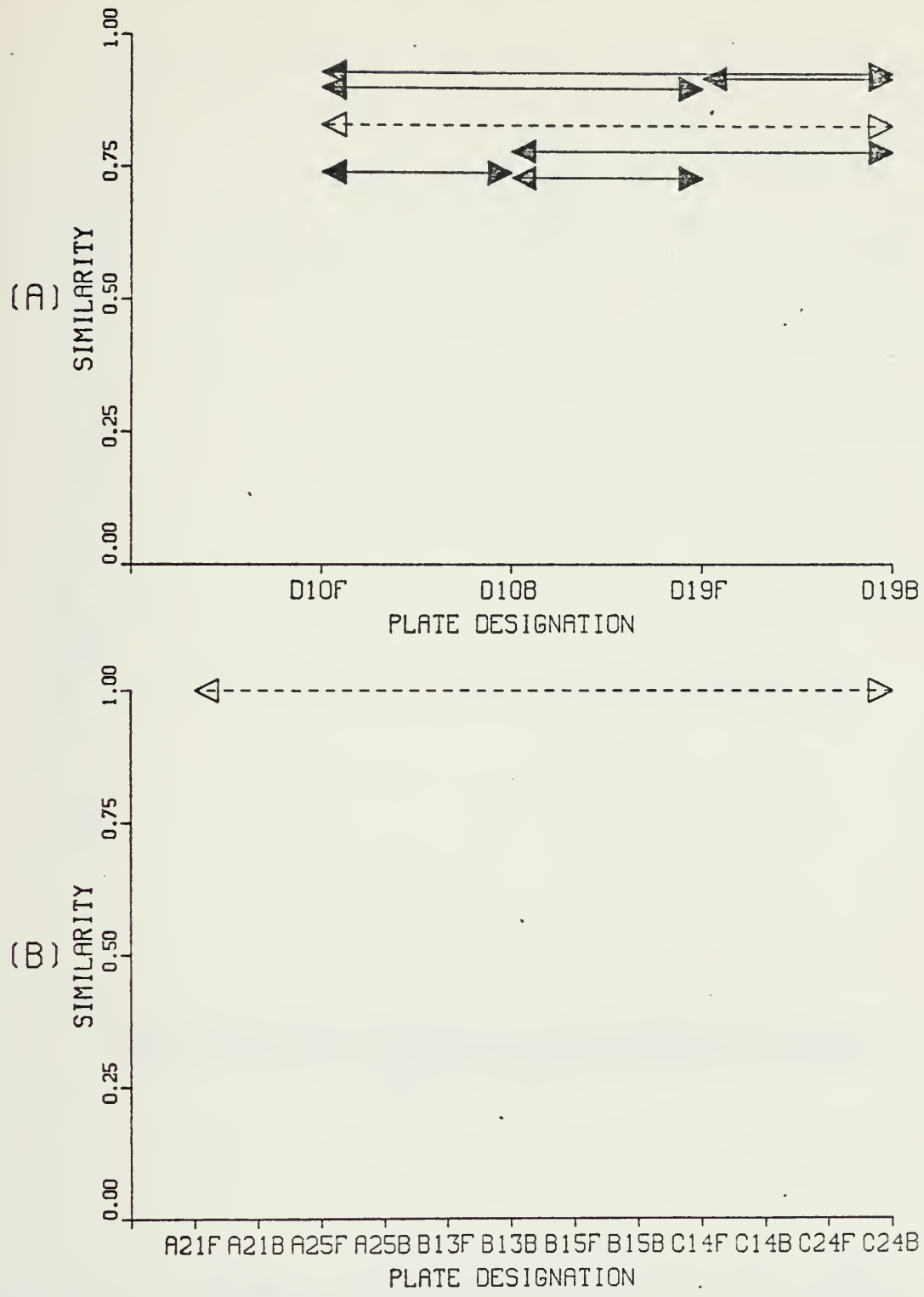

Figure 8. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 2. Dashed Lines Indicate Mean Similarity Values.

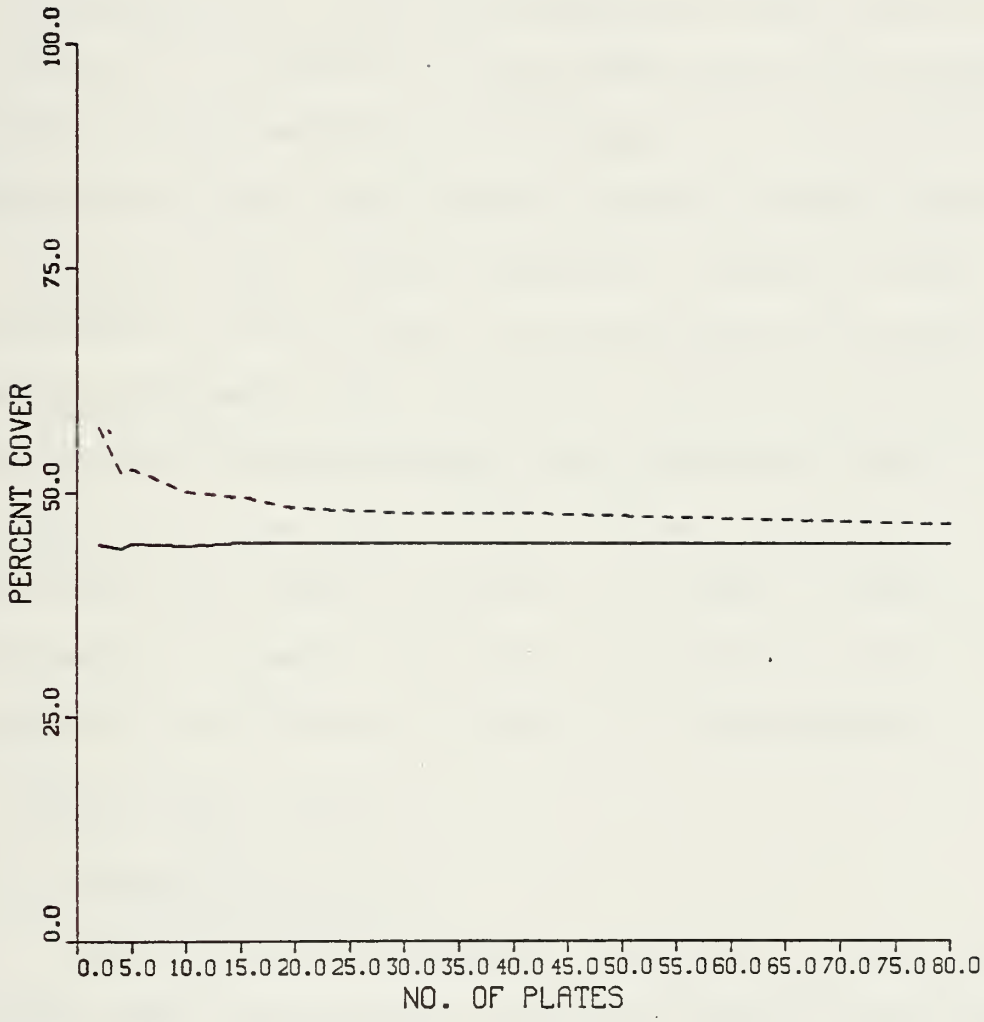

Figure 9. Computer Simulations Using Data From Month 2 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

limit of the percent fouling cover that 90% of the plates would have (Figure 10A). Note that there is very little dependence on N on the percent cover that one can say 90% of the plates will have fouling cover less than or equal to. This is in spite of the fact that the standard deviation that these 90% of the plates will have about the mean percent cover does display a strong inverse dependence on N (Figure 10B). While these two results might seem dichotomous, they are not. The standard deviation about the mean does decrease with increasing sample size just as one would expect from the Central Limit Theorem. The fact that the 95% confidence limit of the fouling cover of 90% of the plates does not behave similarly is simply a result of the fact that by allowing interplate variability, one no longer constrains the parameter to have a 1/ N dependence.

C. RESULTS FROM MONTH 3

1. Experimental Data

The fouling community structure on the control surfaces after three months immersion remained dominated by the hydroid Obelia spp. The four species of spirorbid worms that live in Monterey Bay ,particularly Circeis armoricana, were also present. The protozoan Folliculina sp. was present in large numbers on three of the plates. The bryozoans were represented by four species with Celloporaria brunnea dominating this group.

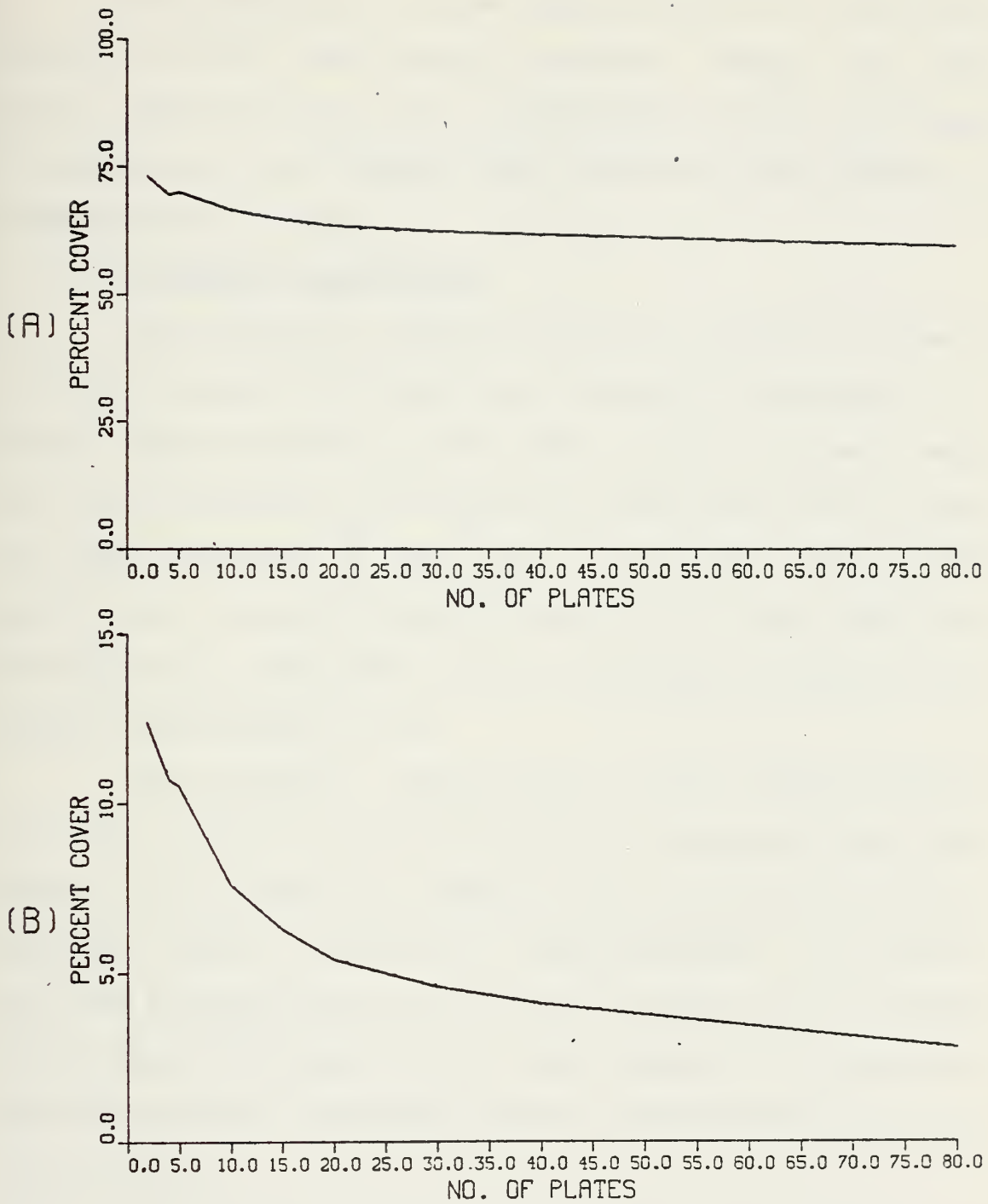

Figure 10. Computer Simulations Using Data From Month 2 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

The similarity values ranged from .57 to .86 with a mean of .74 for the control surfaces (Figure 11A). The mean similarity for the antifouling coated surfaces remained at 1.0 (Figure 11B) since no settlement of organisms had occured.

2. Computer Simulations

The computer simulations of the expected value of the mean percent fouling cover (Figure 12) resulted in a final iterated value of 49.5% vice a 49.3% arithmetic mean of the initial data. The upper 95% confidence limit about the mean decreased by nearly 8% as the number of simulated plates per group increased from 2 to 15 and then only decreased an additional 3% as the number of plates per group was increased to 80.

The computer simulations of the 95% confidence limit of the fouling cover that 90% of the plates would be fouled to an equal or lesser extent showed the value remained nearly constant at approximately 70% (Figure 13A).The standard deviation of the percent cover on these plates about the mean percent cover showed little relative decrease past the simulated analysis of 20 plates per group (Figure 13B).

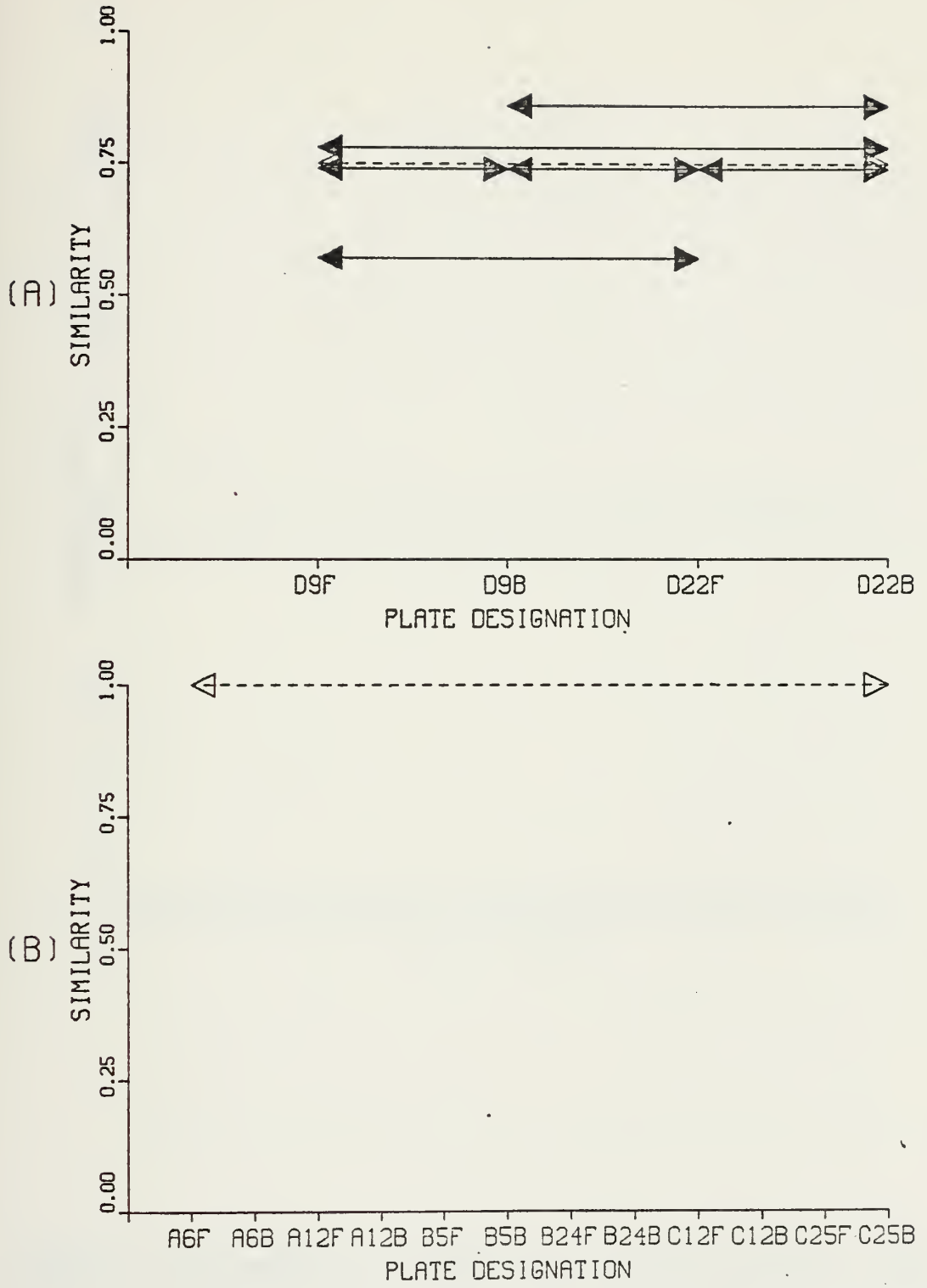

Figure 11. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 3. Dashed Lines Indicate Mean Similarity Values.

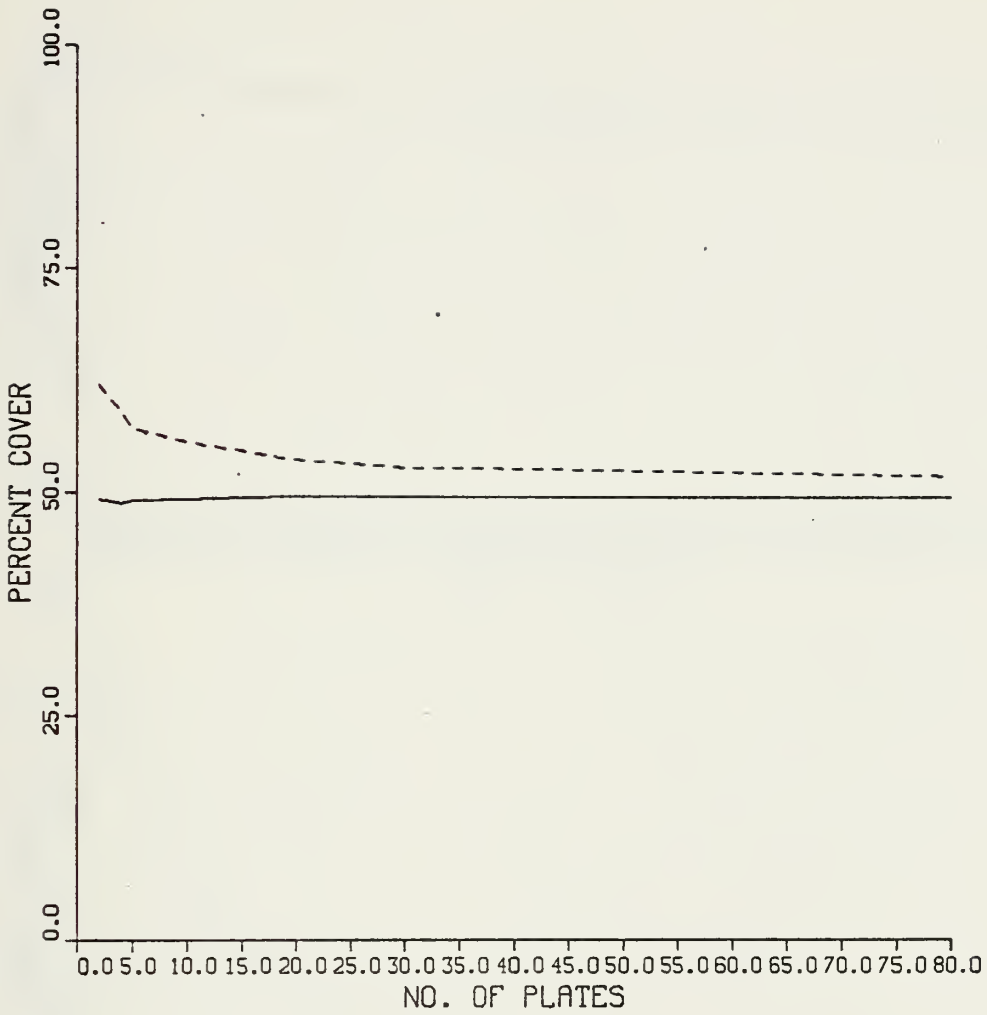

Figure 12. Computer Simulations Using Data From Month 3 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

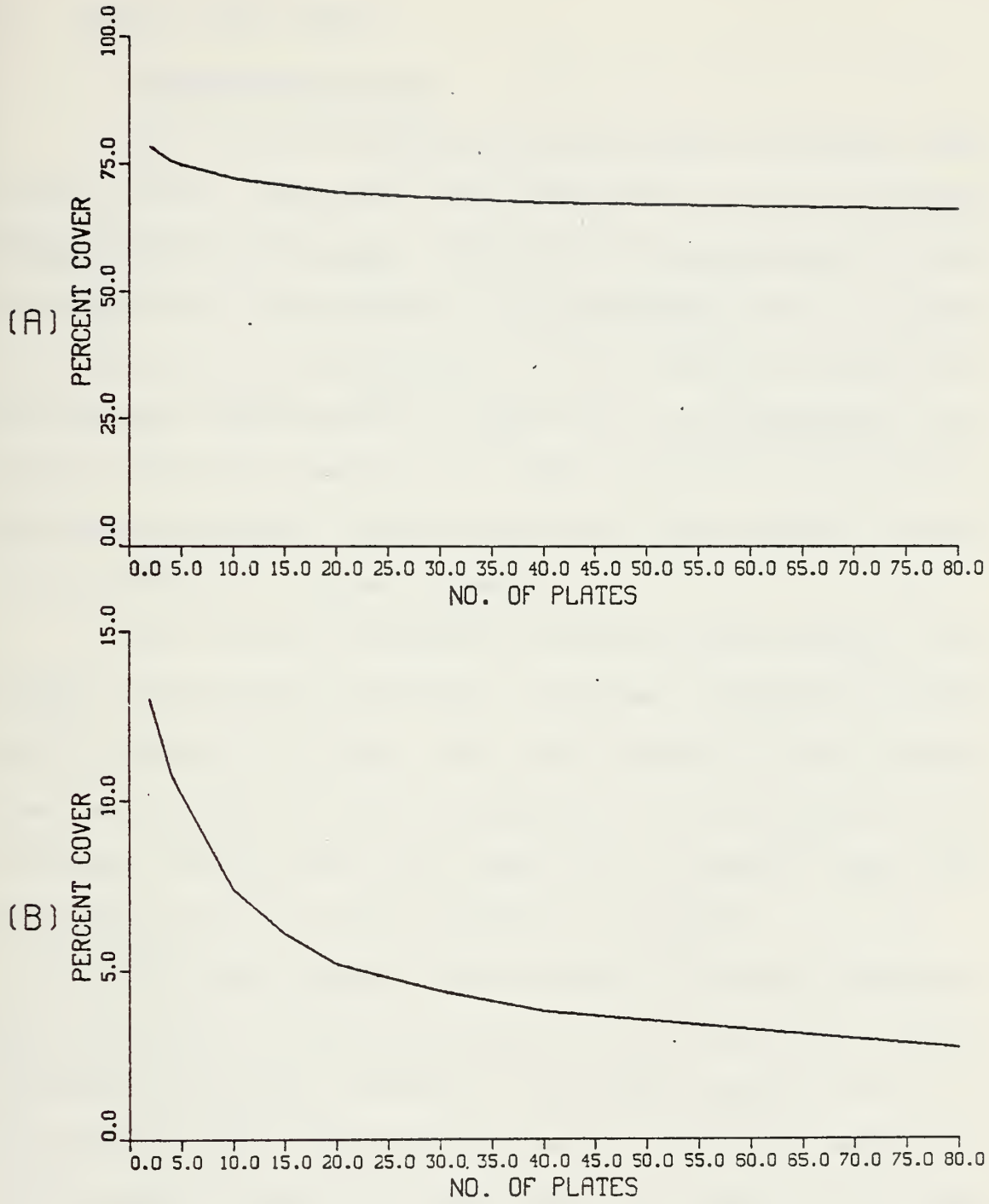

Figure 13. Computer Simulations Using Data From Month 3 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

D. RESULTS FROM MONTH 4

1. Experimental Data

The fouling community on the control surfaces after four months immersion was dominated by the bryozoan Watersipora cuculatta. The hydroid Obelia spp. had been overgrown to a large extent by W. cuculatta and as a result its relative contribution to the percent cover was diminished considerably. In addition to W. cuculatta, there were six other species of either upright or encrusting bryozoans present. Various spirorbid and serpulid worms were present in limited numbers.

The percent coverage estimates varied greatly from plate to plate with a maximum of 84% and a minimum estimate on one plate of 2% cover. The wide variability was perhaps partially caused by the ascendency of the bryozoans as the dominant organism but the reason for the nearly total lack of fouling on two of the surfaces is unknown.

The wide range in the percent coverage estimates was mirrored in the variability of the similarity indices between the control surfaces. The values ranged from .16 to .98 (Figure 14A). The antifouling coated plates still had no fouling so again the mean similarity was 1.0 for those plates.

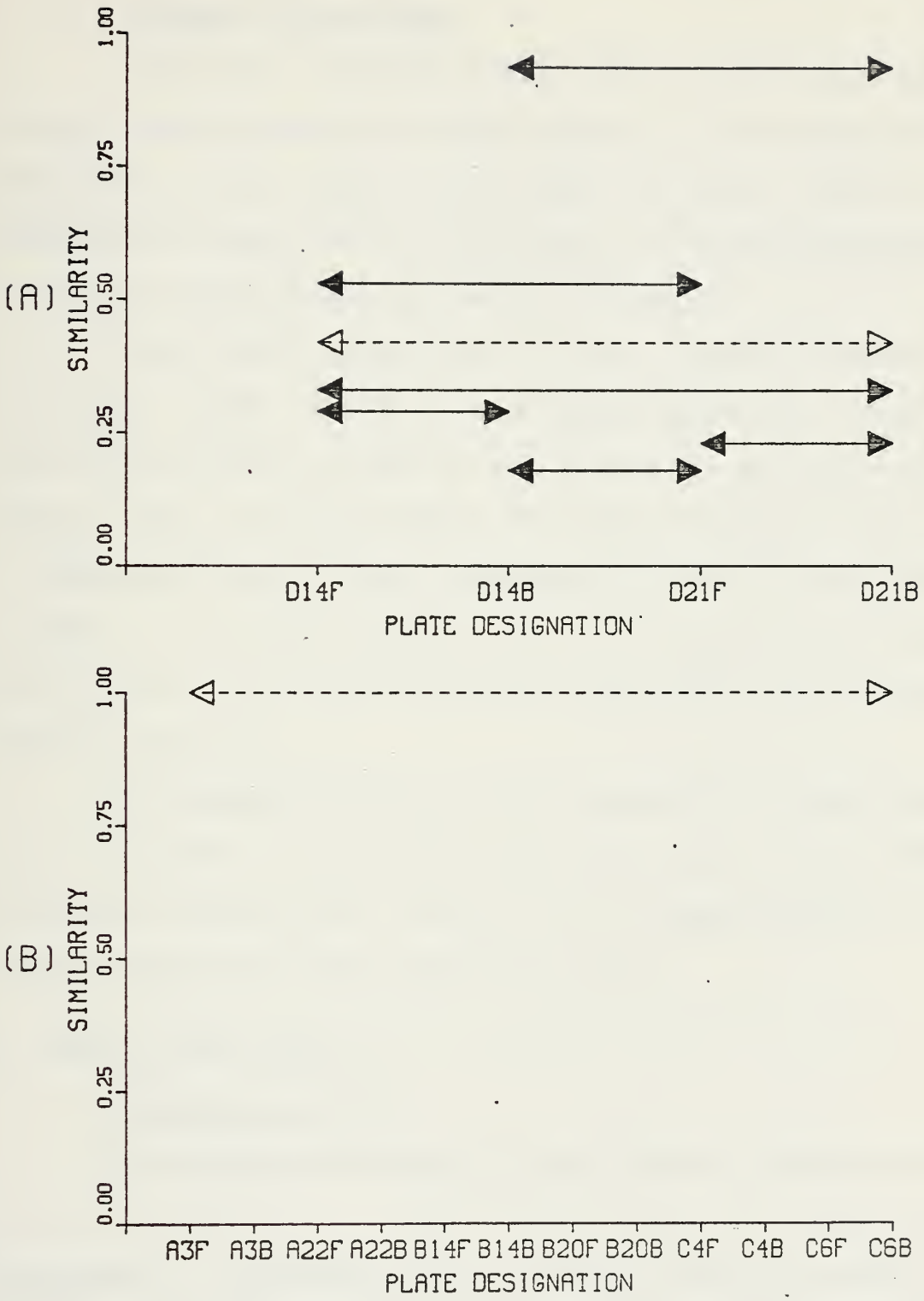

Figure 14. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 4. Dashed Lines Indicate Mean Similarity Values.

2. Computer Simulations

The final iterated value for the simulated mean percent fouling cover was 31% (Figure 15). The upper 95% confidence limit about this mean was quite large and decreased by nearly 40% as the number of simulated plates per group went from two to twenty (Figure 15).

The wide variability of the percent coverage estimates caused the 95% confidence about the fouling coverage estimate of 90% of the plates to be quite high (Figure 16A). As can be seen, the only statement that can be made about the fouling coverage that 90% of the plates willhave is that the coverage will be something less than 93% (Figure 16A) even though the estimated mean percent coverage was 31%.

The standard deviation that these 90% of the plates will have about the mean was also quite large (Figure 16B) and showed significant decline out to approximately the thirty plates per group simulation point.

E. RESULTS FROM MONTH 5

1. Experimental Data

The bryozoan dominance of the fouling community was firmly established by the fifth month of immersion. Watersipora cuculatta was the primary fouler on three of the four control surfaces and there were an additional

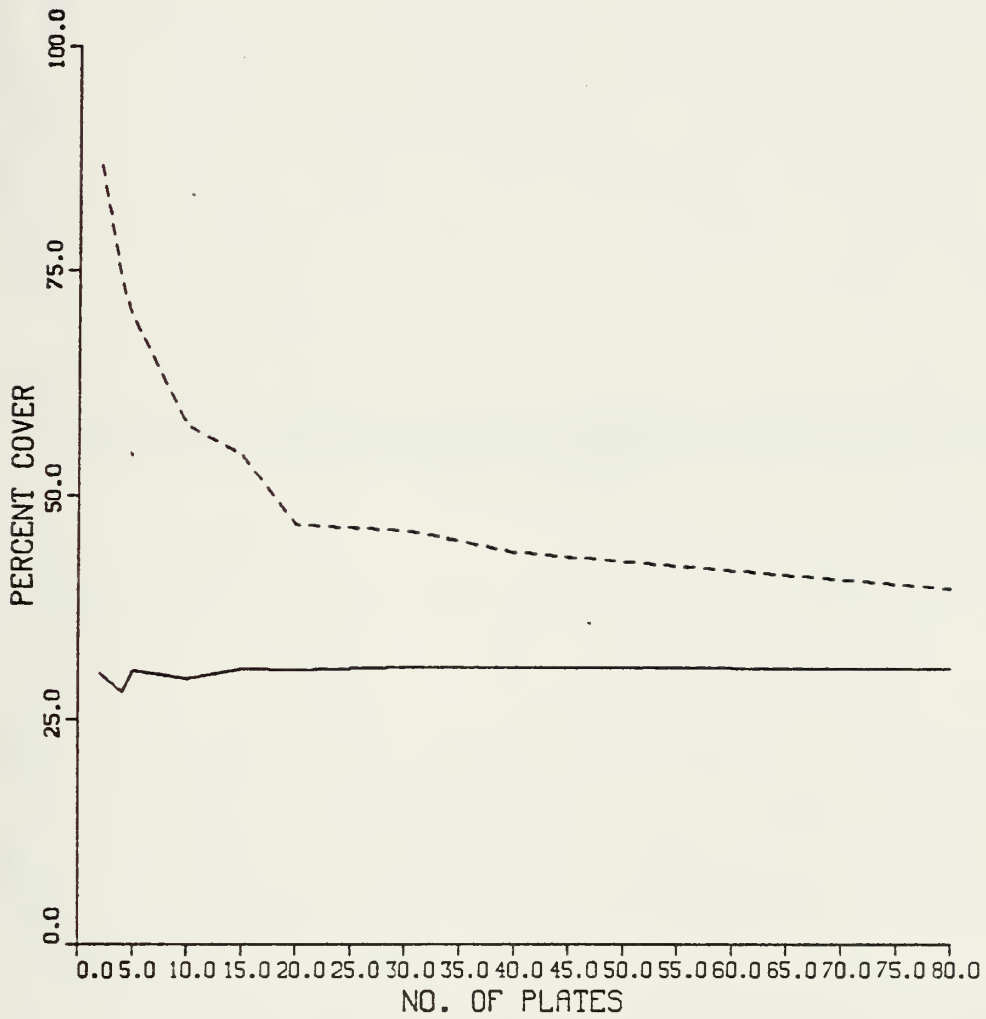

Figure 15. Computer Simulations Using Data From Month 4 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

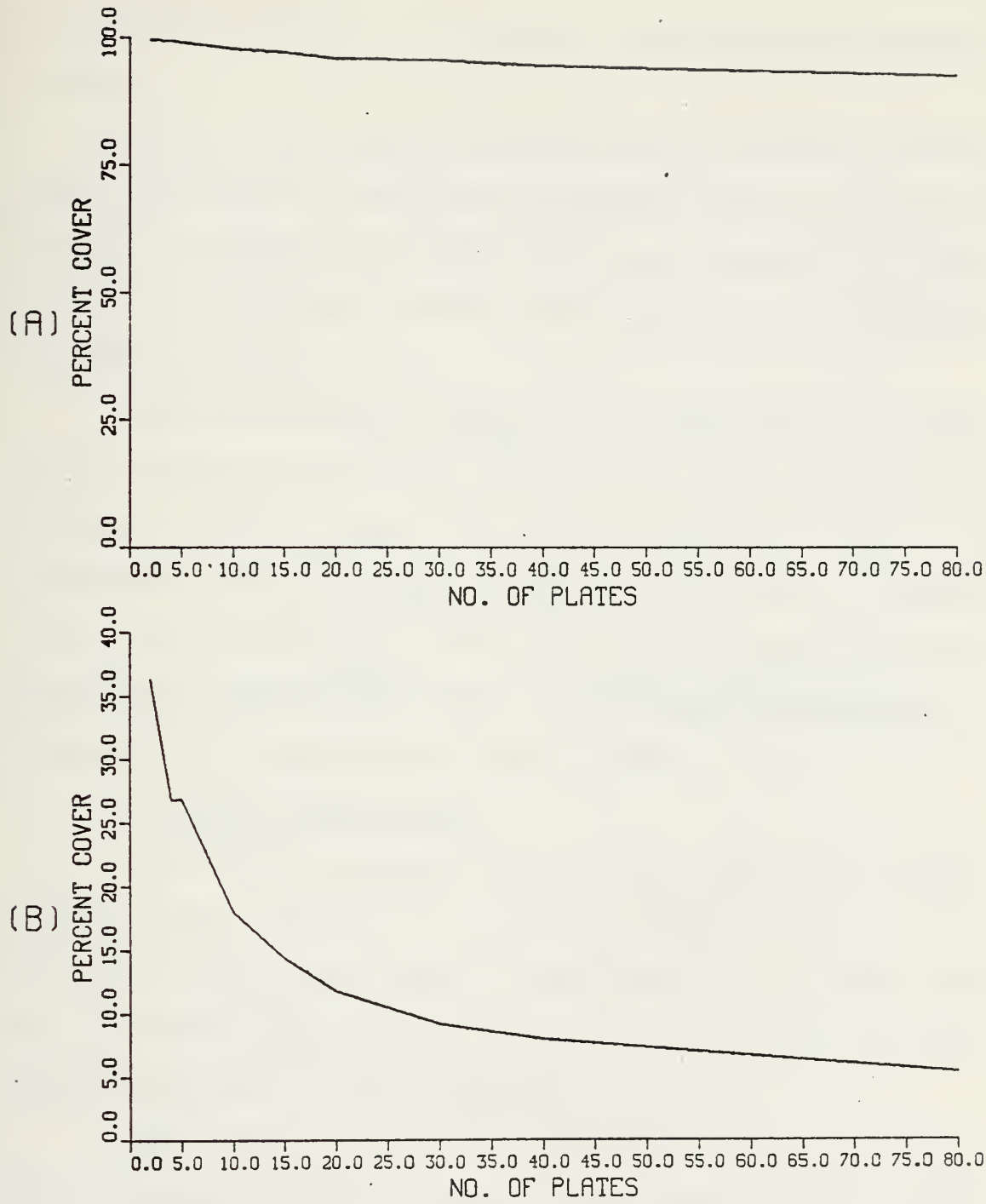

Figure 16. Computer Simulations Using Data From Month 4 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

eight other species of bryozoans identified during the census.

There was a fairly large range of initial percent cover estimates for the control surfaces with a minimum of 13% and a maximum of 96%. The mean percent coverage estimate for these plates was 63% and the standard deviation was 31%.

The similarity indices for the control surface plates ranged from .17 to .67 with a mean value of .45 (Figure 17A). The similarity values for the antifouling coated surfaces ranged from .85 to 1.0 (Figure 17B). This variability was caused by the settlement of Obelia spp. and the spirorbid worm Circeis armoricana on several of the antifouling coated plates.

2. Computer Simulations

The final iterated estimate for the mean percent cover was 69% (Figure 18) vice the arithemetic mean of 63% for the initial data. The 95% coinfidence limit about this mean showed an appreciable decrease out to approximately twenty plates per group simulated.

The population variability again resulted in the 95% confidence limit of the fouling cover of 90% of the plates having virtually no dependence of the number of samples analyzed (Figure 19A). The standard deviation about the mean that these 90% of the plates would have showed

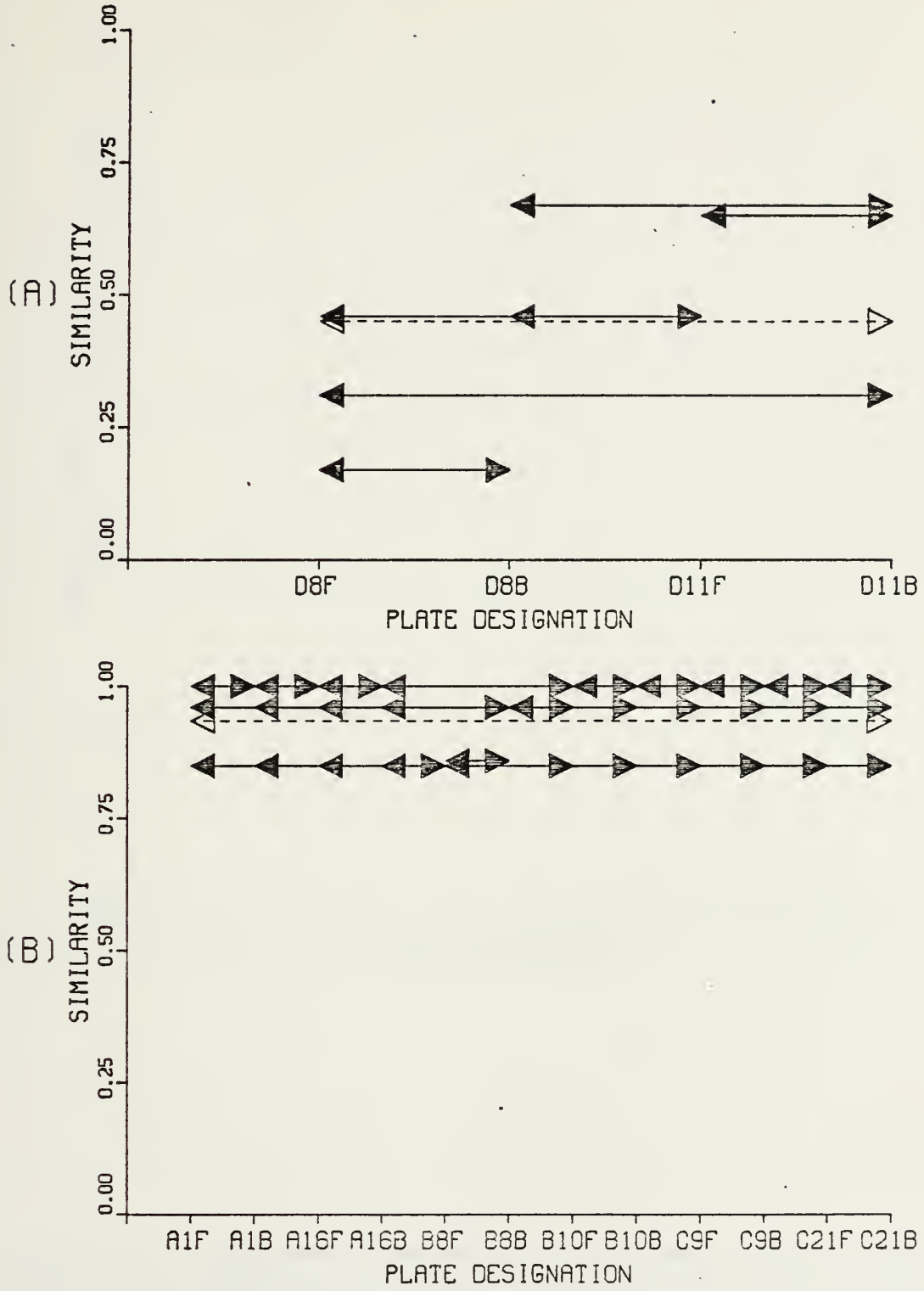

Figure 17. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 5. Dashed Lines Indicate Mean Similarity Values.

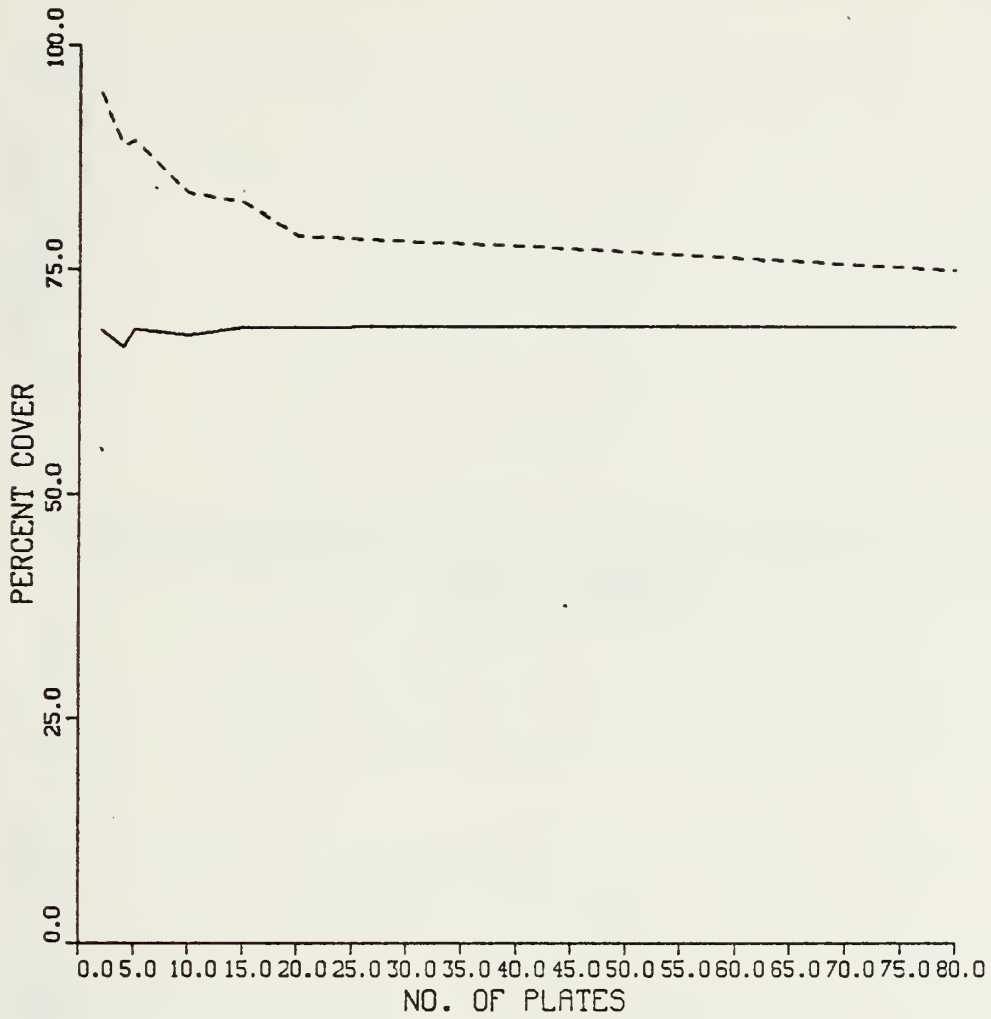

Figure 18. Computer Simulations Using Data From Month 5 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

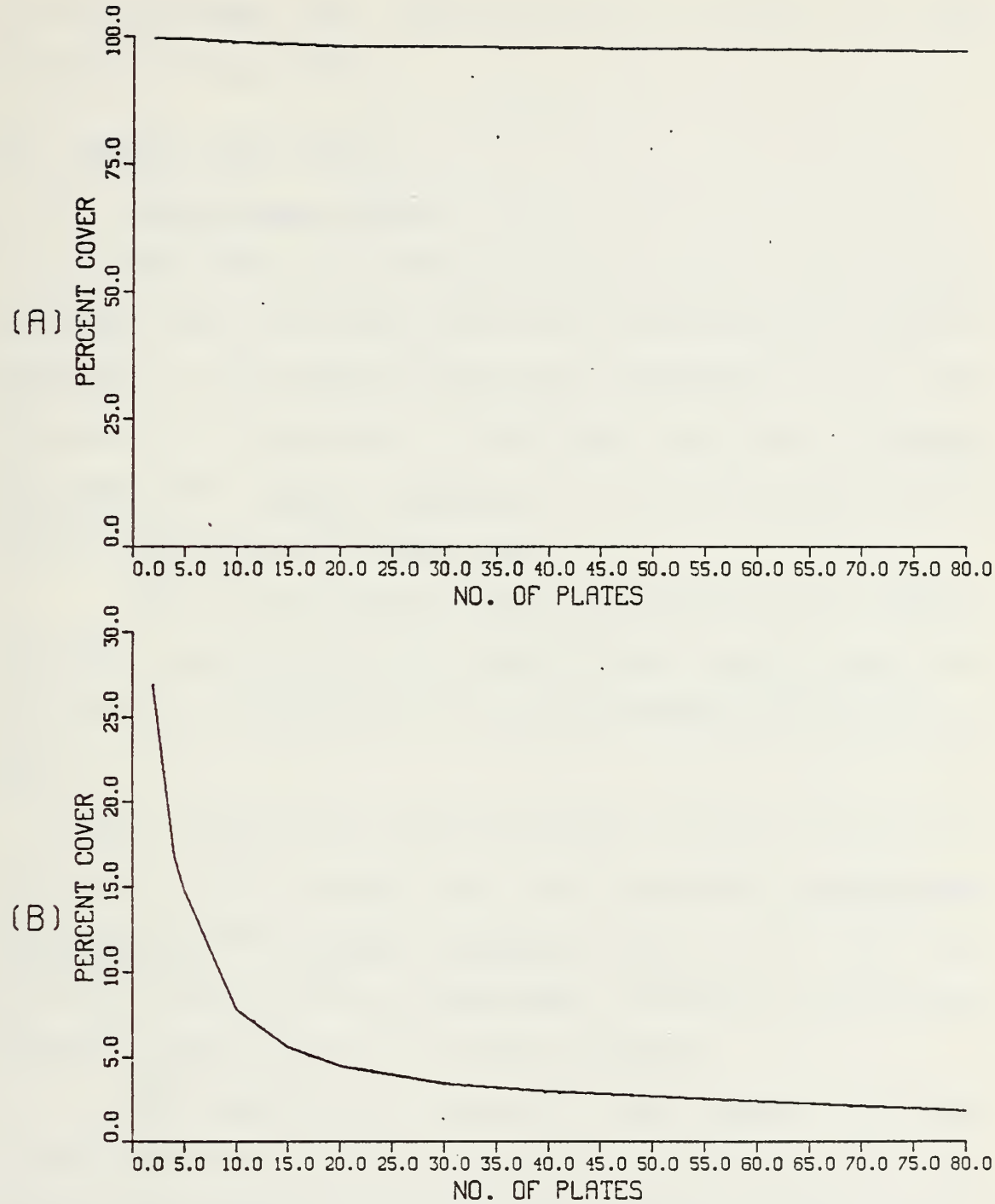

Figure 19. Computer Simulations Using Data From Month 5 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

little decrease past approximately 20 plates per group simulated (Figure 19B).

F. RESULTS FROM MONTH 6

1. Experimental Data

The fouling community structure on the control surfaces after six months immersion was dominated either by bryozoans or the hydroid Obelia spp. depending on the plate examined. Two species of solitary tunicates, Ascidia ceretodes and Styela truncata were also censused for the first time during the experiment.

The initial percent cover estimates for the control surfaces ranged from 16% to 54%. The mean percent cover for the control surfaces was 37% with a standard deviation of 18%.

The antifouling coated surfaces showed fouling on several of the plates with the barnacles Megabalanus californicus and Balanus crenatus appearing for the first time on these surfaces. Obelia spp. remained the dominant fouler on the antifouling coated surfaces.

The similarity values for the control surfaces ranged from .48 to .88 with a mean of .70 (Figure 20A). The mean similarity of the antifouling coated surfaces was .99 (Figure 20B).

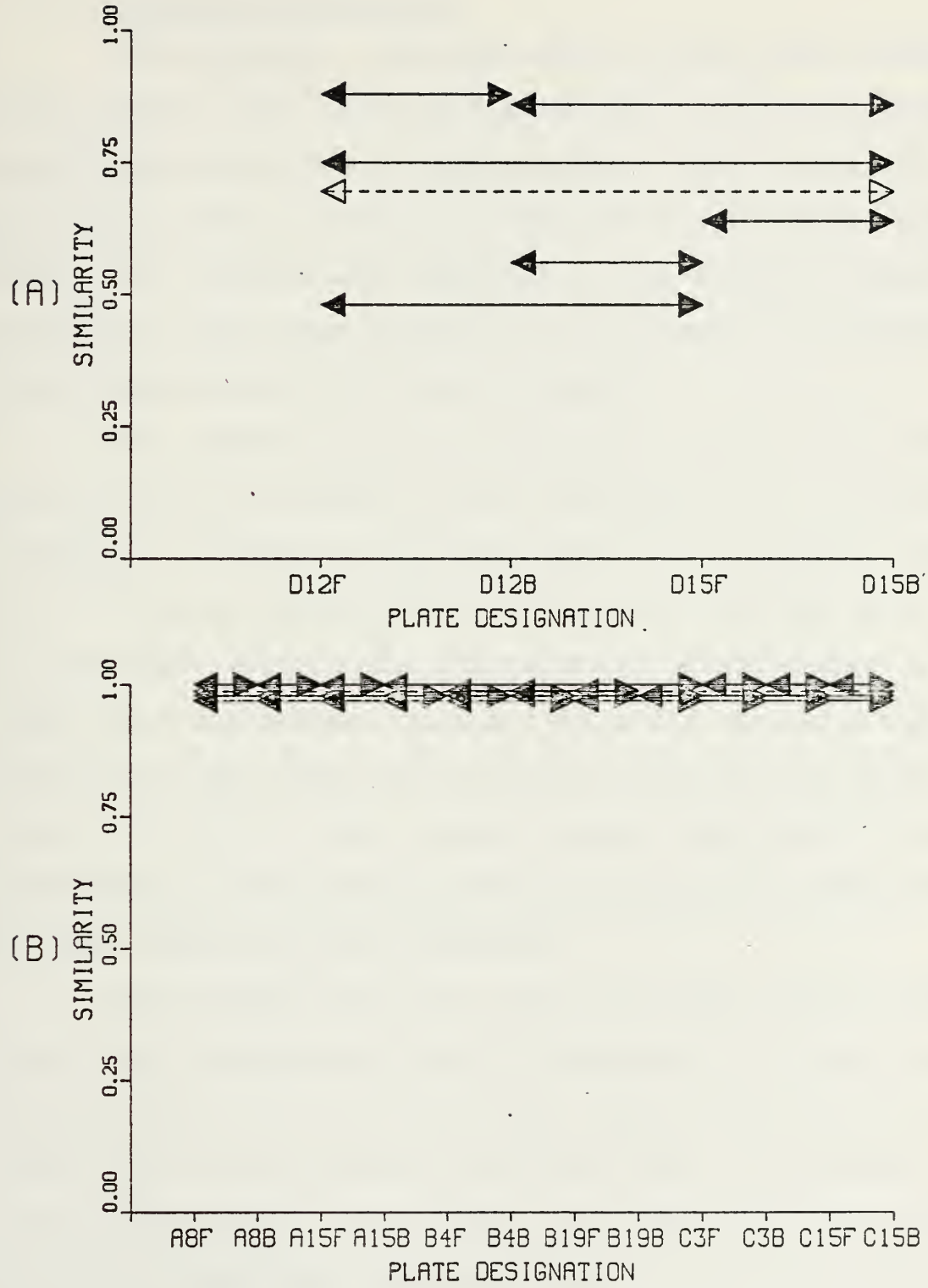

Figure 20. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 6. Dashed Lines Indicate Mean Similarity Values.

2. Computer Simulations

The computer simulated value of the mean percent cover was 33%. The upper 95% confidence limit about the mean showed significant decrease out to approximately 20 plates per group simulated (Figure 21). Note again that there was a significant decrease in the width of the 95% confidence limit about the mean as the number of plates per group simulated went from ten to twenty.

The rather high mean similarity value for the sixth month control plates and by inferrence the lessened interplate variability permitted the 95% confidence limit of the fouling cover of 90% of the plates (Figure 22A) to be less than that of the fourth month simulations even though the mean percent coverage value for the sixth month group was higher. The 95% confidence limit of the fouling cover of 90% of the plates value decreased until approximately the twenty plates per group simulation point and then remained fairly constant.

The standard deviation about the mean percent cover that these simulated plates displayed decreased only slightly past the 20 plates per group simulated point (Figure 22B). Note however that there was a 5% decrease in this value between the 10 plates per group simulated point and the 20 plates per group simulated.

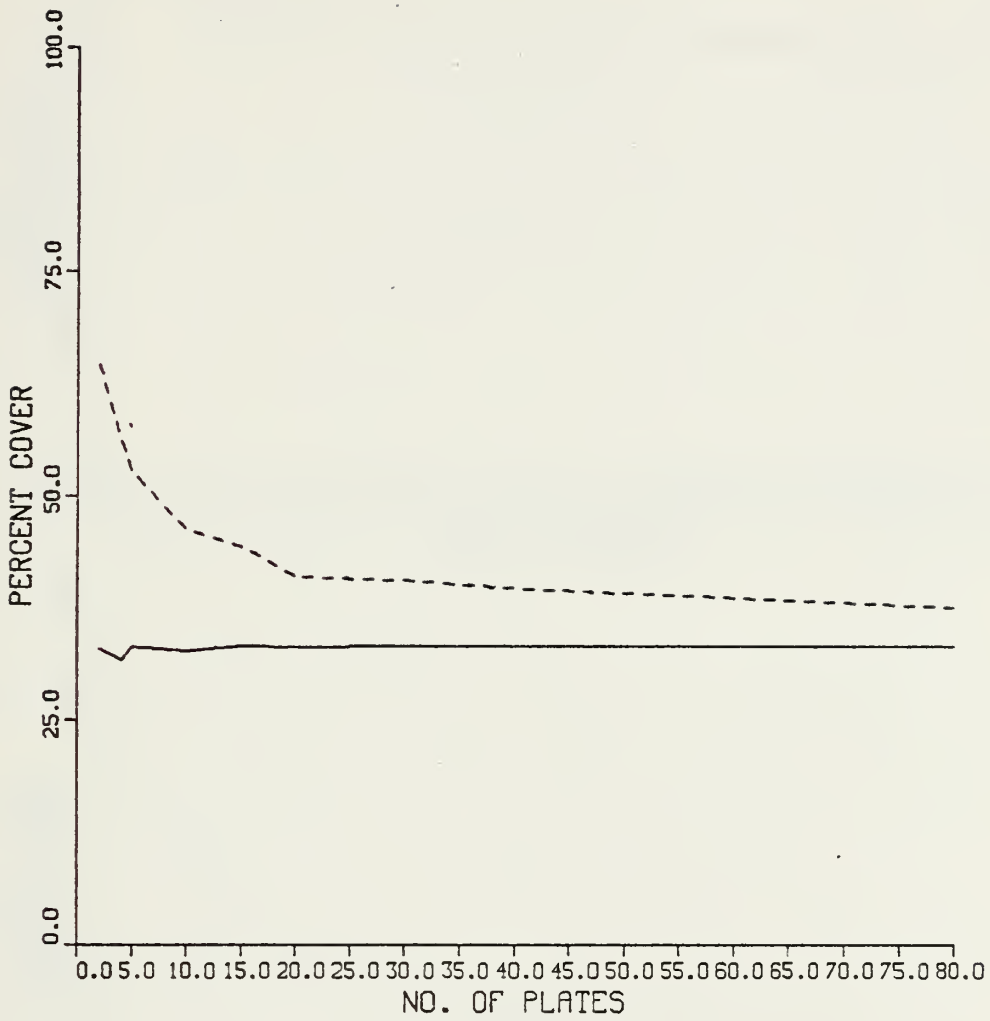

Figure 21. Computer Simulations Using Data From Month 6 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

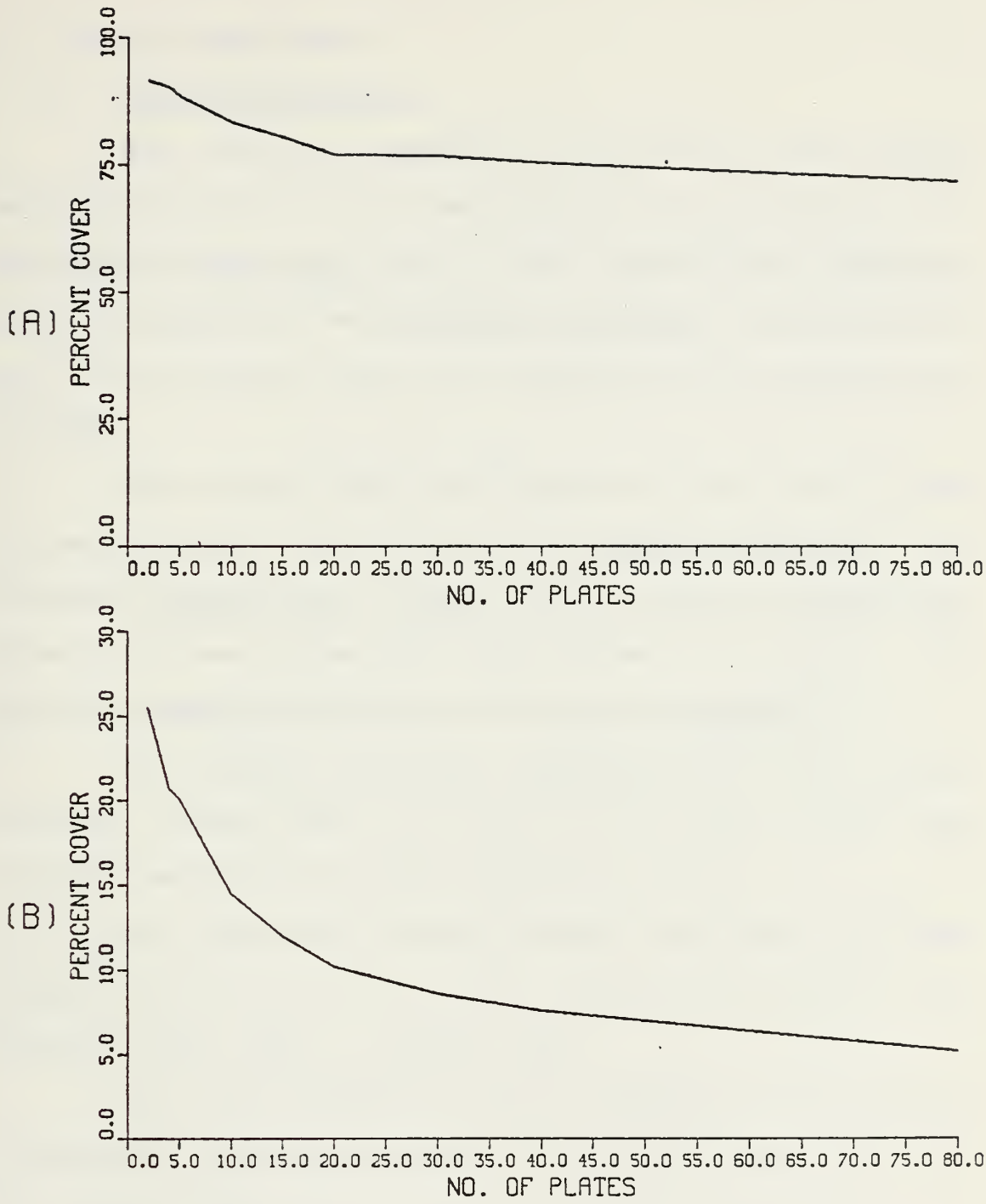

Figure 22. Computer Simulations Using Data From Month 6 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

G. RESULTS FROM MONTH 7

1. Experimental Data

The fouling community structure of the seventh month conrol plates remained dominated by bryozoans with the hydroid Obelia spp. still in an important but secondary role. The spirorbid worm Circeis armoricana was dominant on one of the plates and was common on all the control surfaces.

The primary fouling organisms on the antifouling coated surfaces were the barnacle Balanus crenatus , the bryozoan Celloporaria brunnea ,and the hydroid Obelia spp. Several of these plates were also fouled by an unknown tube dwelling amphipod, possibly of the genus Ampithoe.

The percent coverage estimates for the control surfaces ranged from 31% to 62% with a mean of 49%. The standard deviation of these values was 11.5%.

The similarity indices between the control surfaces ranged from .22 to .88. The mean similarity value for the control plates was .55 (Figure 23A).

For the antifouling coated surfaces, the range of similarity values was from .81 to 1.0. The mean value was .92 (Figure 23B).

2. Computer Simulations

The final iterated value for the simulated mean percent fouling coverage was 49%. The upper 95% confidence

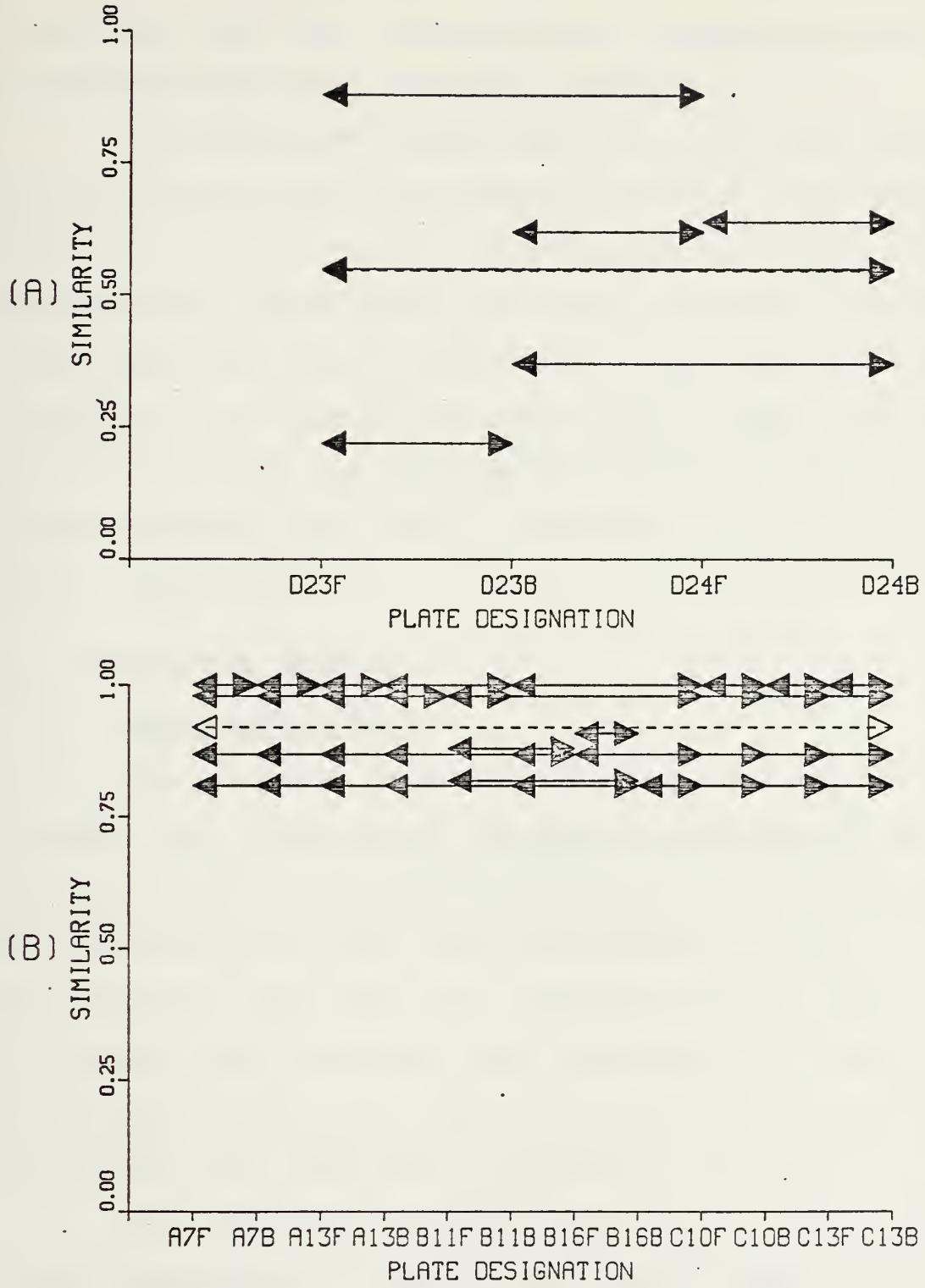

Figure 23. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 7. Dashed Lines Indicate Mean Similarity Values.

limit about this mean remained fairly constant past the 20 plates per group simulated point (Figure 24).

The simulated percent cover that one could say 90% of the plates would have fouling coverage less than or equal to (Figure 25A), decreased slightly until approximately the 20 plates per group simulation point and then remained fairly constant at approximately 65% coverage. The standard deviation that these simulated plates had about the mean percent fouling cover showed little decrease past the 30 plates per group simulation point (Figure 25B).

H. RESULTS FROM MONTH 8

1. Experimental Data

The fouling community structure on the control surfaces after eight months immersion was dominated nearly exclusively by bryozoans. The contribution of hydroids to the percentage of cover had been reduced on three of the surfaces to less than 5%. Serpulid worms, solitary tunicates, and barnacles were beginning to emerge as important foulers as their increasingly large size prohibited their overgrowth by encrusting species.

The fouling organisms present on the antifouling coated surfaces were primarily the hydroid Obelia spp. and barnacles. The spirorbid worm Circeis armoricana was also present on several of the plates.

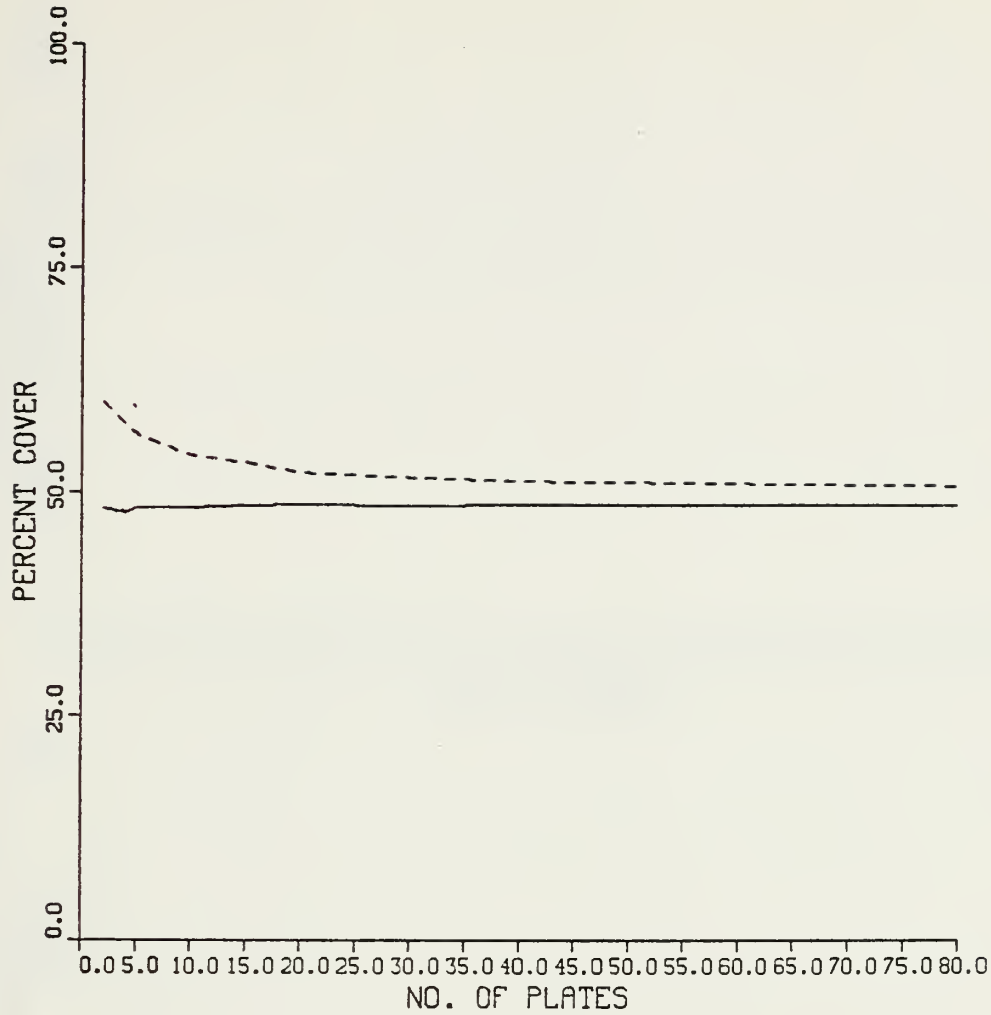

Figure 24. Computer Simulations Using Data From Month 7 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

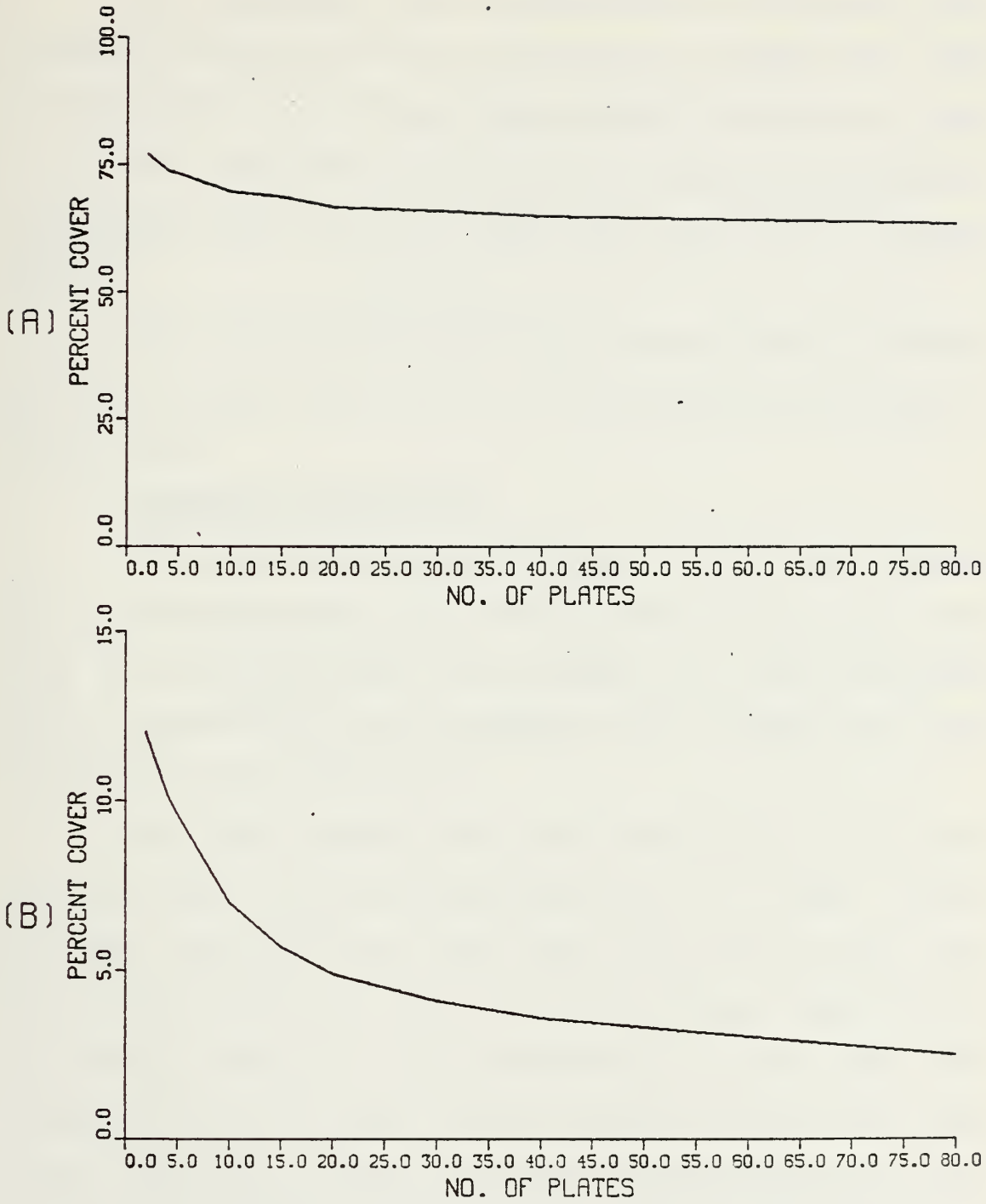

Figure 25. Computer Simulations Using Data From Month 7 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

The estimates for the percentage of fouling cover ranged from 48% to 97% for the control surfaces. The mean of these values was 76% and the standard deviation was 19%.

For the control surfaces, the range of similarity values was from .22 to .67. The mean value was .44 (Figure 26A).

The similarity values for the antifouling coated surfaces ranged from .75 to 1.0. The mean value was .94 (Figure 26B).

2. Computer Simulations

The computer iterated estimate for the mean percent fouling coverage for the control surfaces was 81%. Note that the 95% confidence limit about this value remained fairly constant past approximately the 25 plates per group simulation point (Figure 27).

The high estimate for the simulated mean percent cover and the variability permitted by the model forced the upper 95% confidence limit about the percent cover that 90% of the plates would have to remain greater than 96% for the entire range of the simulations (Figure 28A). The standard deviation about the mean percent cover that these plates would have showed a precipitous decrease out to the 20 plates per group simulated point and then decreased only 2% out to the 80 plates per group simulation point (Figure 28B).

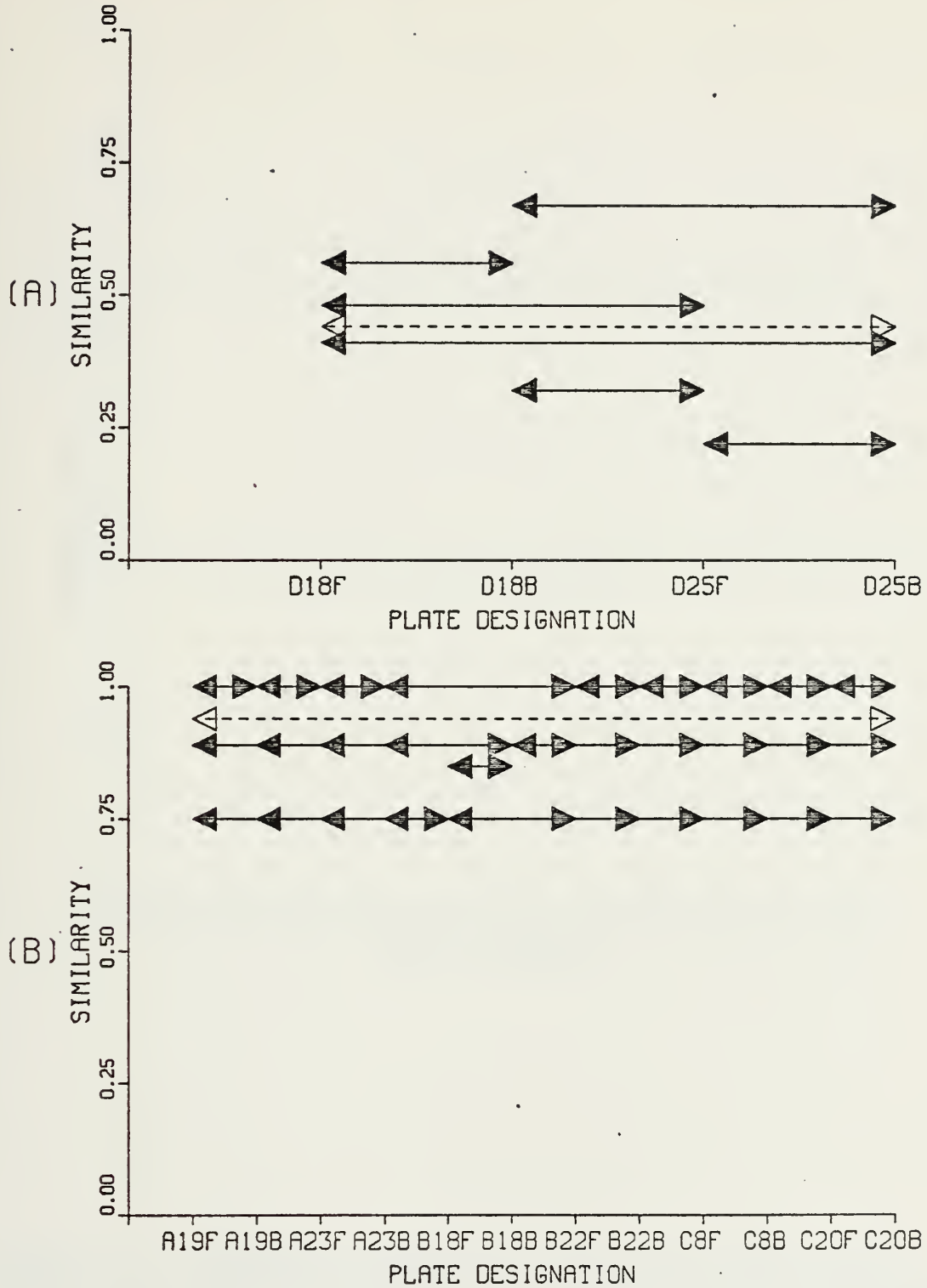

Figure 26. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 8. Dashed Lines Indicate Mean Similarity Values.

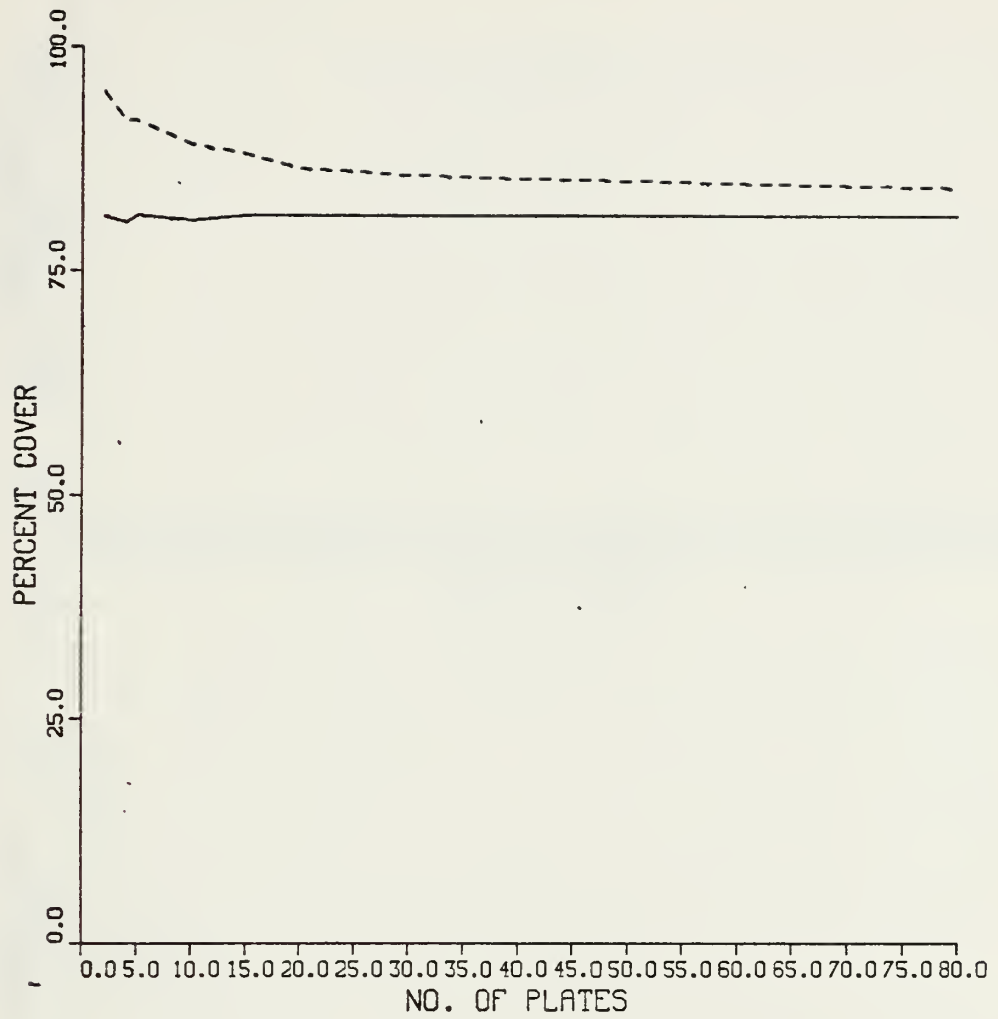

Figure 27. Computer Simulations Using Data From Month 8 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

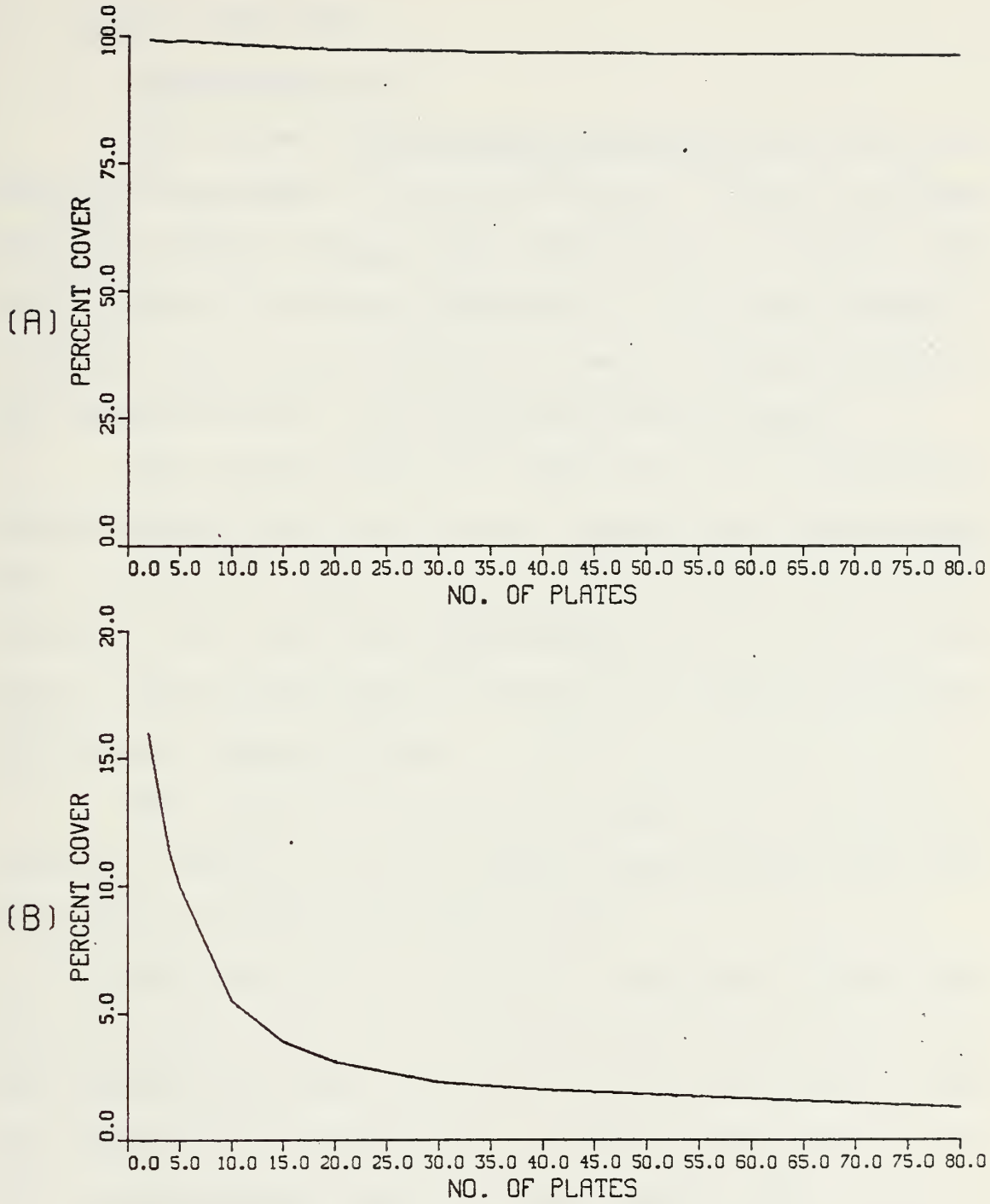

Figure 28. Computer Simulations Using Data From Month 8 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

I. RESULTS FROM MONTH 9

1. Experimental Data

The bryozoan Watersipora cuculatta dominated the fouling assemblages on three of the four control surfaces while the hydroid Obelia spp. dominated on the fourth. The total number of species represented in the census began to stabilize as the more successful species excluded others in the competition for the rapidly diminishing space.

The dominant fouling organism on the antifouling coated surfaces was the bryozoan, Membranipora membranacea. This organism is usually found nearly exclusively on the fronds of the giant kelp Macrocystis and it is not known what chemical or other stimulus attracted it to the antifouling coated surfaces.

The percent coverage estimates for the control surfaces ranged from 35% to 92%. The mean percent cover was 67% and the standard deviation was 25%.

The similarity values for the plates ranged from .18 to .74 for the control surfaces and from .60 to 1.0 for the antifouling coated surfaces. The mean similarity value for the control surfaces was .41 (Figure 29A) and was .89 for the antifouling coated surfaces (Figure 29B).

2. Computer Simulations

The final estimate for the mean percent fouling cover for the computer simulated plates was 73%. The upper

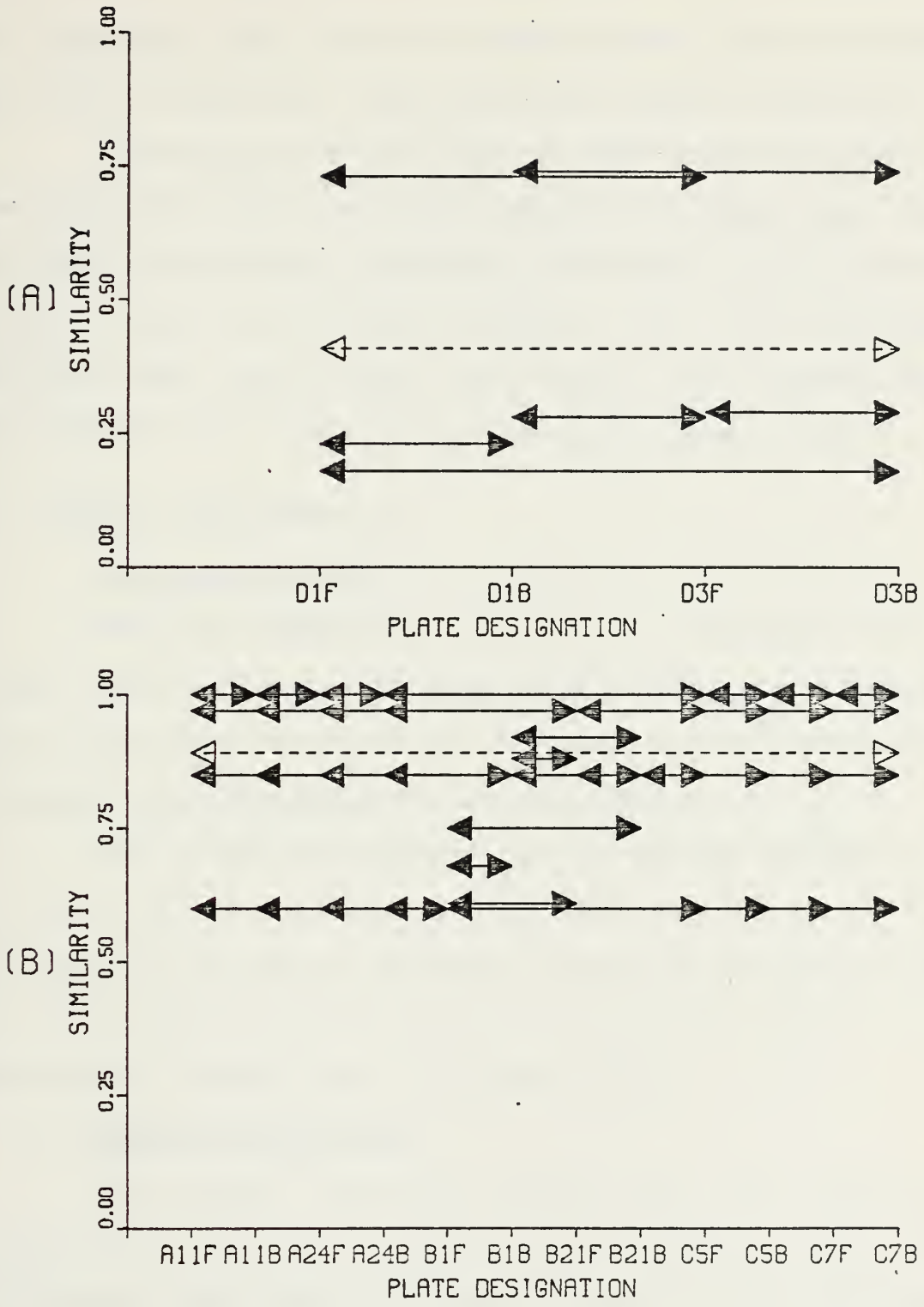

Figure 29. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 9. Dashed Lines Indicate Mean Similarity Values.

95% confidence limit about this mean showed little decrease past the 20 plates per group simulation point (Figure 30).

The 95% confidence limit on what one can say 90% of the simulated plates will have fouling coverage less than or equal to remained above 95% coverage for the entire range of the simulations (Figure 31A). The standard deviation that these plates had showed little change past the 20 plates per group simulation point (Figure 31B).

J. RESULTS FROM MONTH 10

1. Experimental Data

Bryozoans dominated the fouling assemblages on all of the control plates with Watersipora cuculatta occupying 60% of the space on one of the surfaces. Eleven species of bryozoans were identified during the census.

The similarity values for the plates ranged from .20 to .79 for the control surfaces and from .67 to 1.0 for the antifouling coated surfaces. The mean similarity value for the control surfaces was .47 (Figure 32A) and for the antifouling surfaces was .90 (Figure 32B).

2. Computer Simulations

The final iterated value for the computer simulation of the mean percent cover was 70%. The upper 95% confidence limit about this mean showed little decrease past the 20 plates per group simulation point (Figure 33).

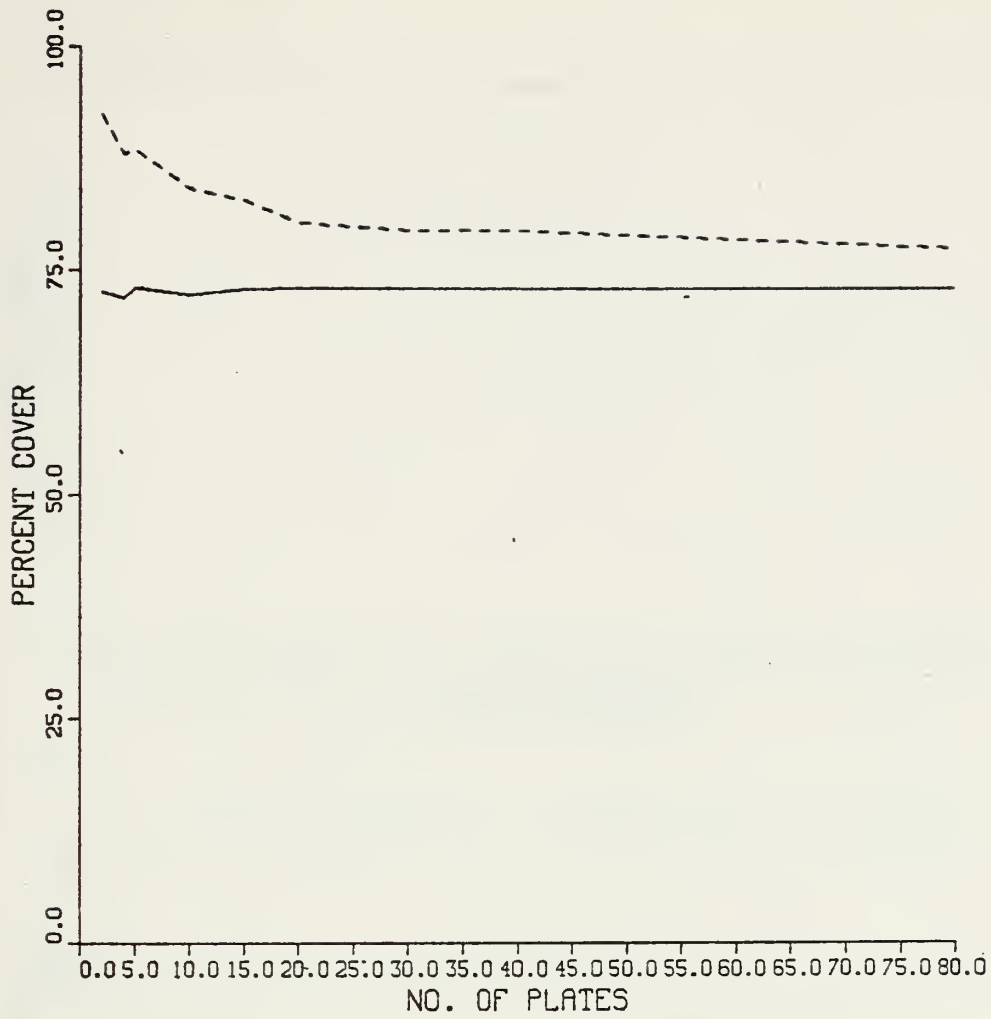

Figure 30. Computer Simulations Using Data From Month 9 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

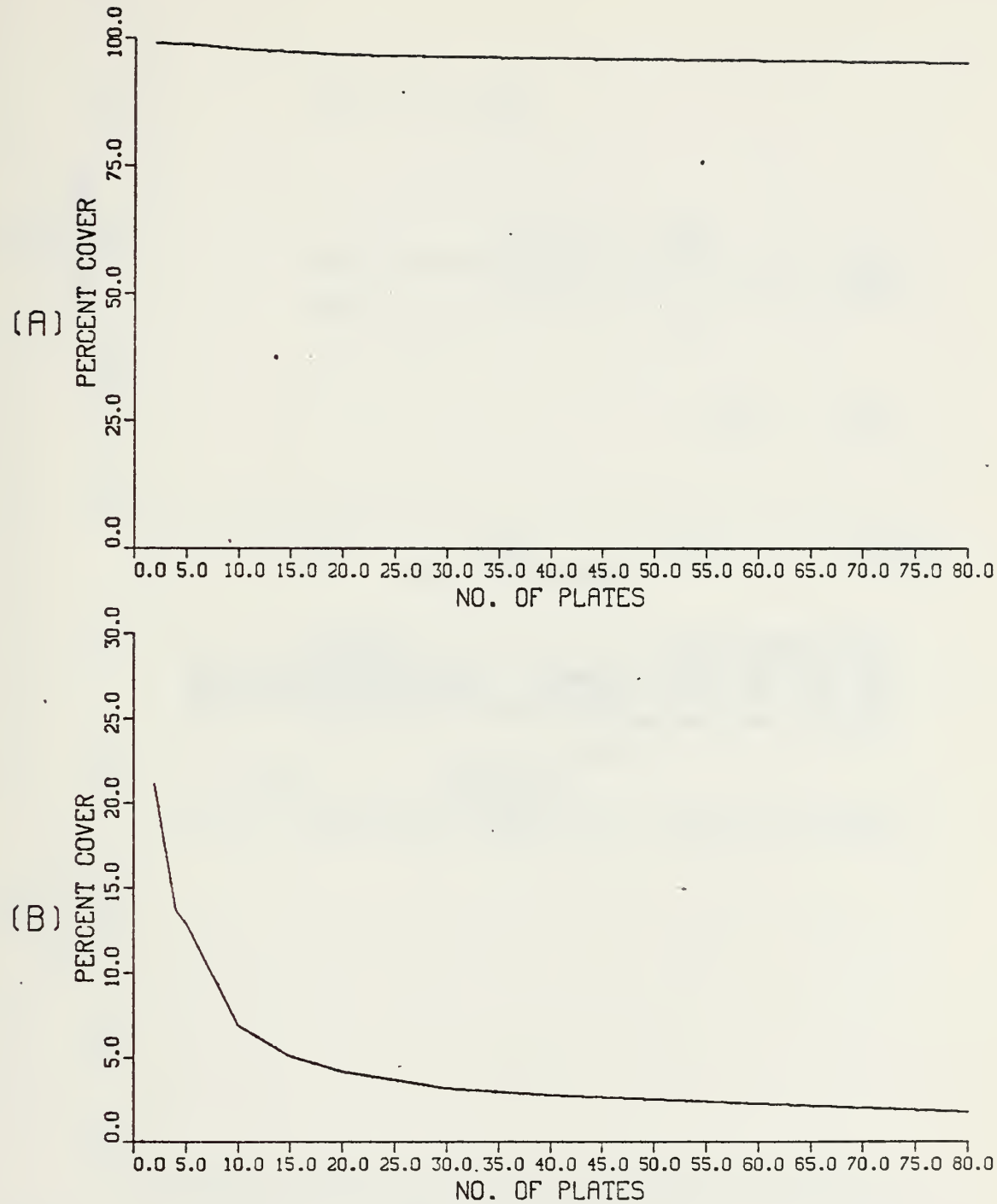

Figure 31. Computer Simulations Using Data From Month 9 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

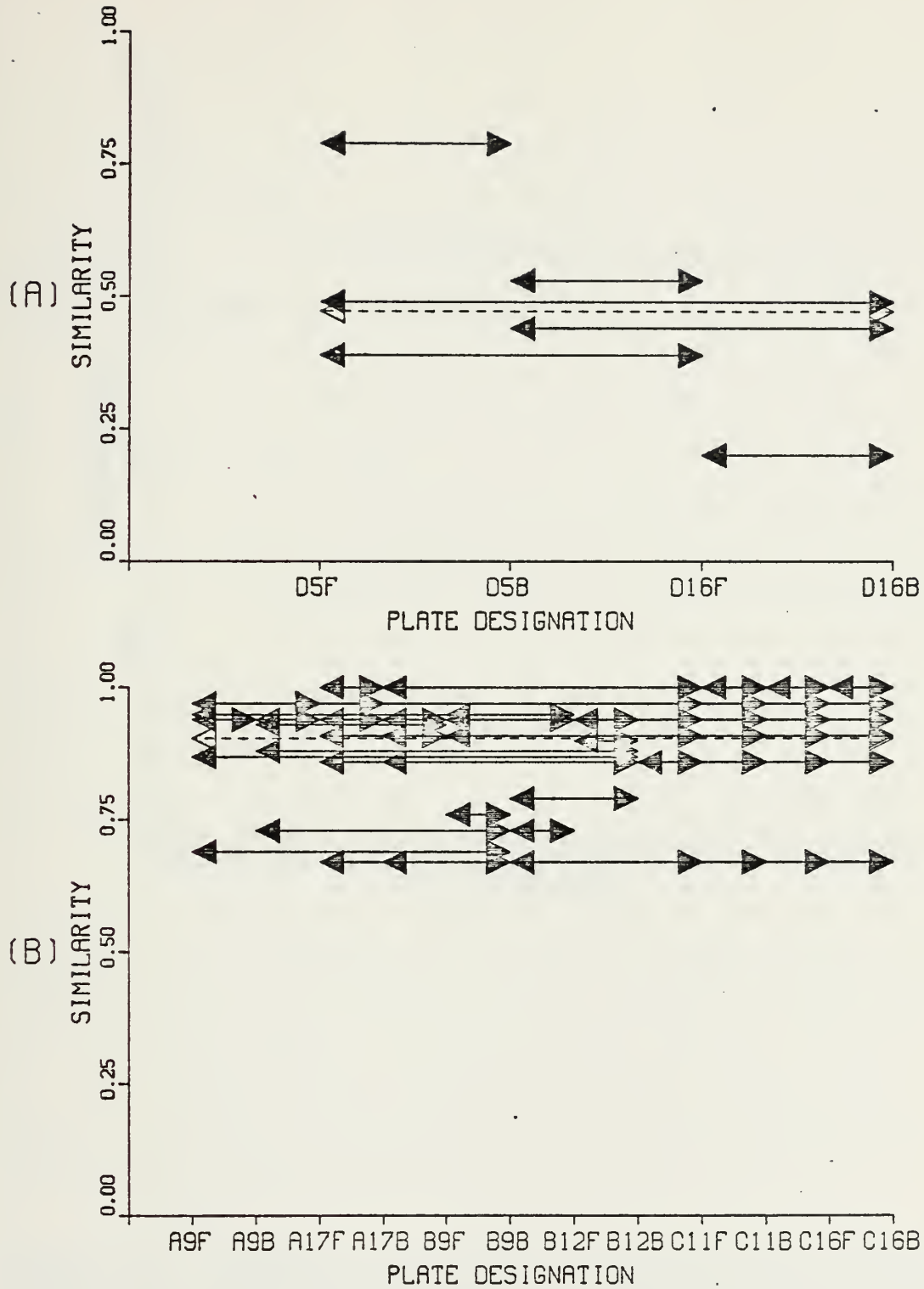

Figure 32. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 10. Dashed Lines Indicate Mean Similarity Values.

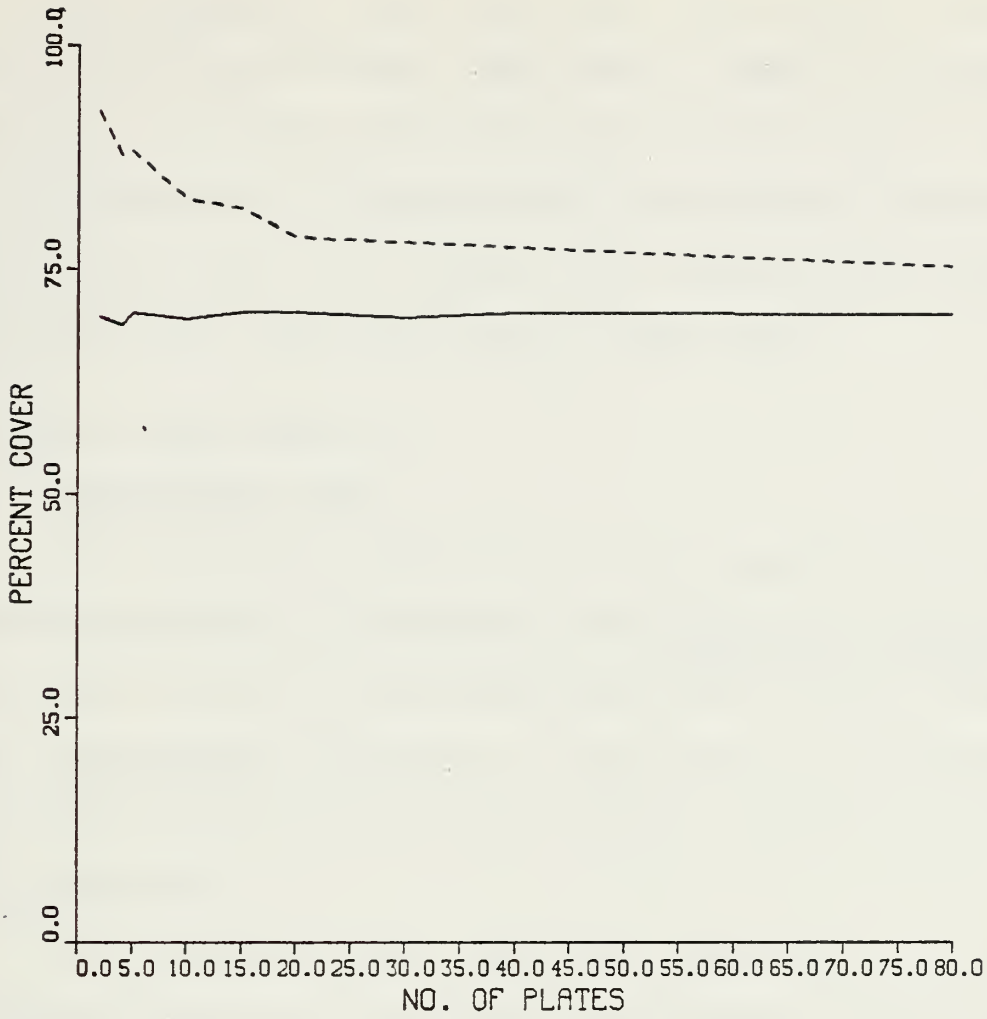

Figure 33. Computer Simulatioms Using Data From Month 10 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

The percent cover that one can say 90% of the plates would have coverage less than or equal to (with 95% confidence) remained above 90% cover for the entire range of the simulations (Figure 34A). The standard deviation these plates would have showed little decrease past the 20 plates per group simulation point (Figure 34B).

K. RESULTS FROM MONTH 11

1. Experimental Data

The fouling communities on the control surfaces remained dominated by bryozoans with Watersipora cuculatta dominating on three of the plates and the upright bryozoan Bugula californica dominating on the fourth. Of the seventeen species identified on these control surfaces, thirteen were bryozoans.

The predominant fouling organism on those antifouling surfaces that showed fouling was the bryozoan Membranipora membranacea. The hydroid Obelia spp. and the spirorbid worm Circeis armoricana were also in evidence.

The initial percent coverage estimates for the control surfaces ranged from 64% to 98%. The mean value was 85% and the standard deviation was 15%.

The similarity values for the plates ranged from .28 to .79 for the control surfaces and from .64 to 1.0 for the antifouling coated surfaces. The mean similarity value

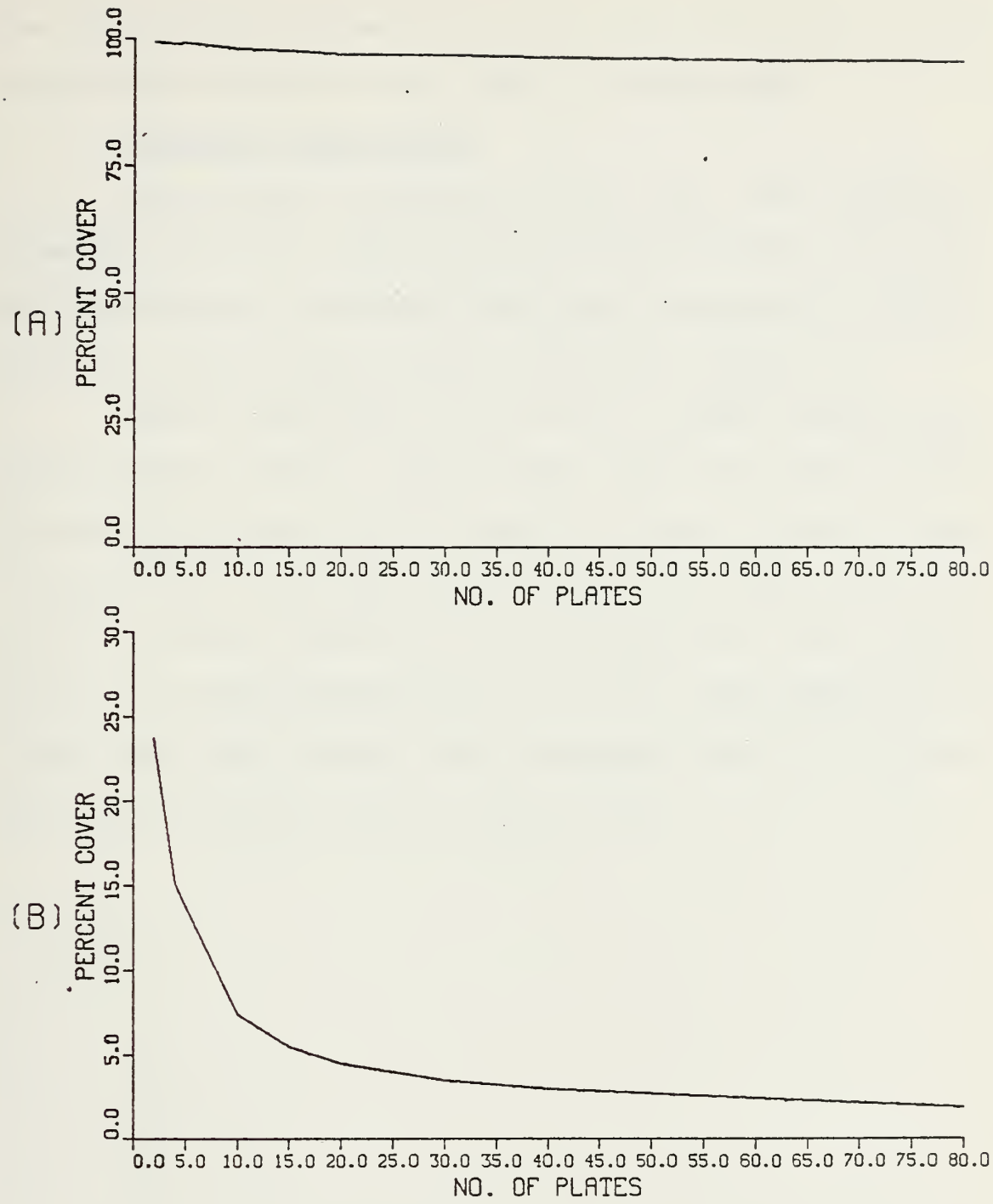

Figure 34. Computer Simulations Using Data From Month 10 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

for the control surfaces was .52 (Figure 35A) and for the antifouling coated surfaces was .87 (Figure 35B).

2. Computer Simulations

The final iterated value for the computer simulations of the mean percent cover was 89%. The upper 95% confidence limit about this mean decreased by only 7% over the entire range of the simulations (Figure 36).

Quite obviously, with such a large valuefor the mean percent cover, the percent cover that 90% of the plates will have will be equally large. As can be seen (Figure 37A), this value never became less that 97% cover over the entire range of the simulations. The standard deviation about the mean percent cover that these plates would have again showed little decrease past the 20 plates per group simulation point (Figure 37B).

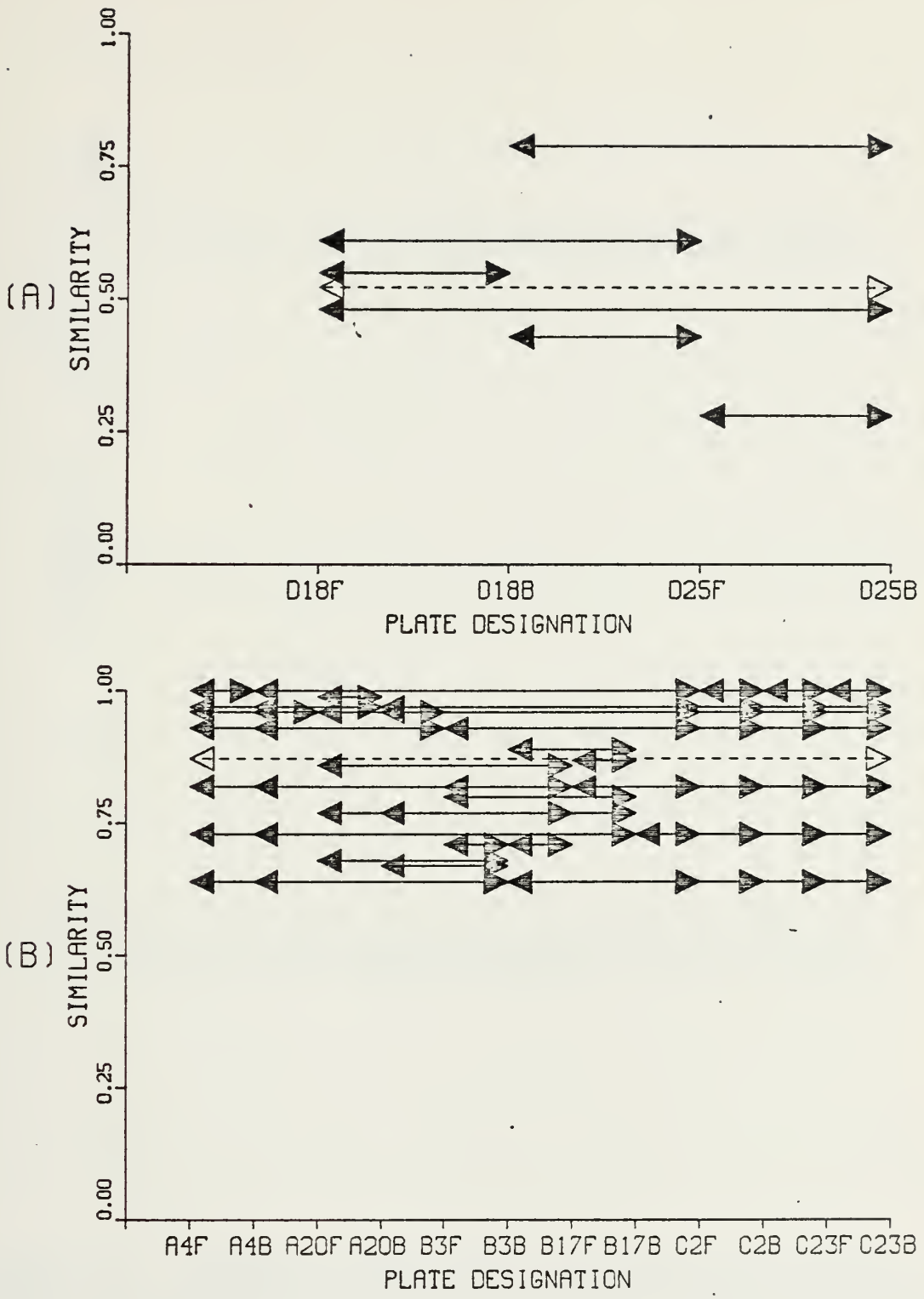

Figure 35. Similarity Graphs for (A) Non-treated Control Surfaces and (B) Anti-fouling Coated Surfaces for Month 11. Dashed Lines Indicate Mean Similarity Values.

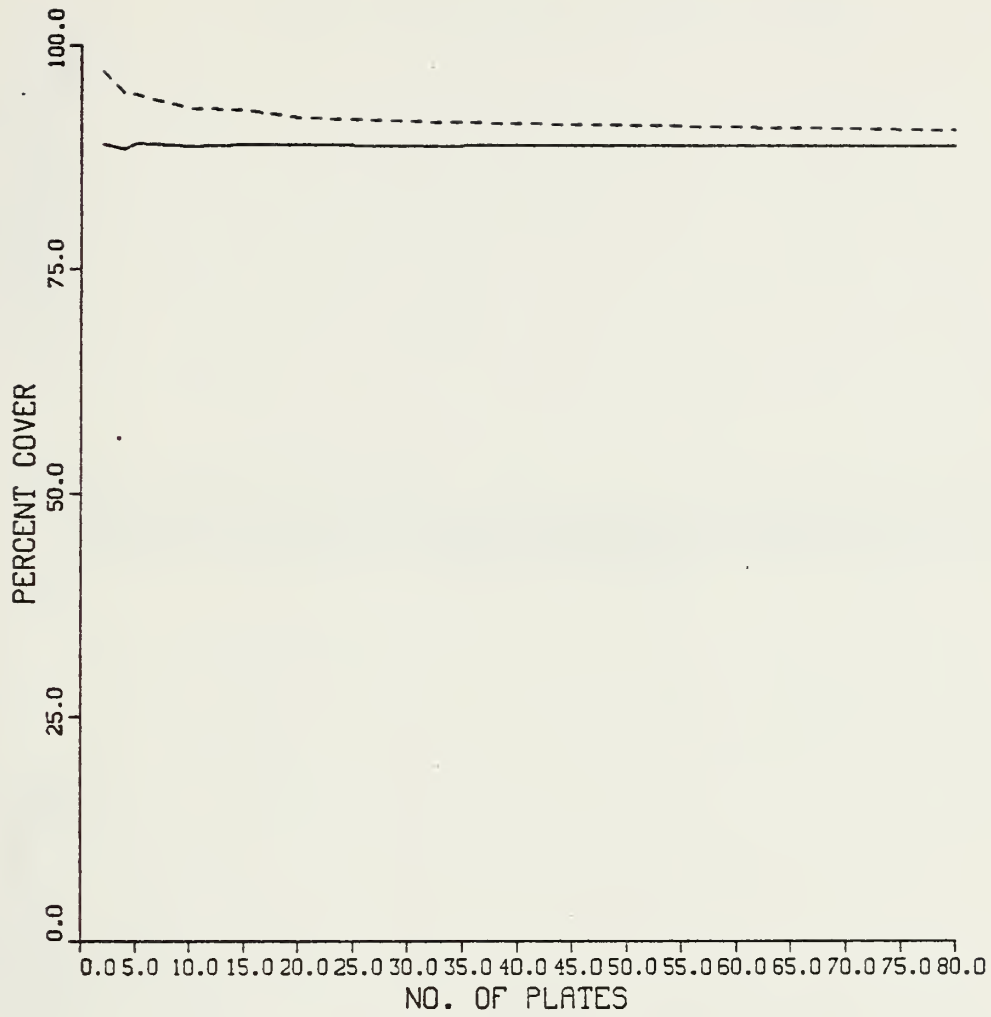

Figure 36. Computer Simulatioms Using Data From Month 11 Showing the Expected Value of the Mean Percent Fouling Cover (the Mean of the 200 Individual Group Simulation Percent Fouling Covers) as a Solid Line and the 95% Quantile (Dashed) of the Expected Mean Percent Fouling Cover as a Function of Increasing Sample Size.

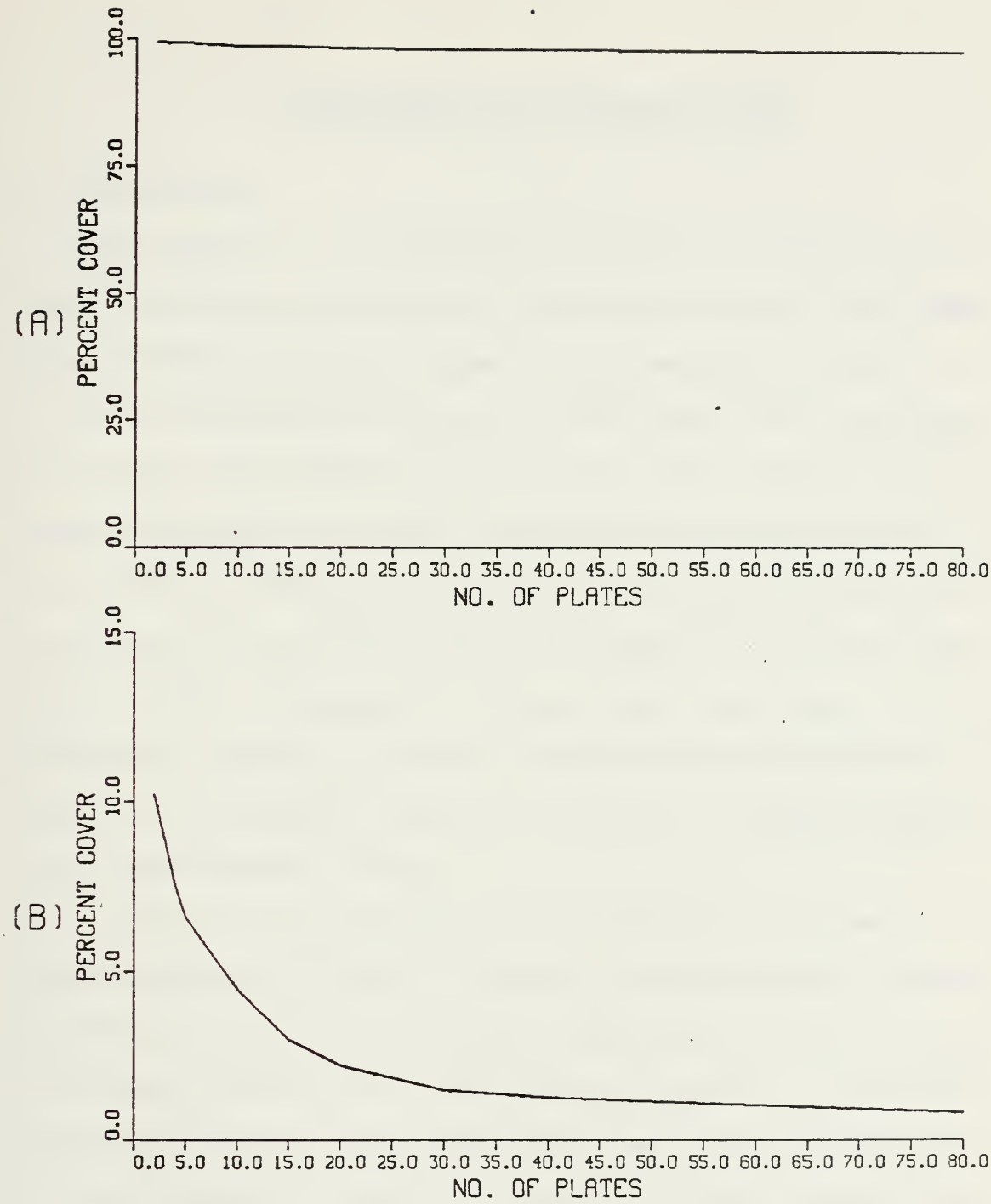

Figure 37. Computer Simulations Using Data From Month 11 Showing : (A) the Percent Fouling Coverage That One Can Say 90% of the Samples Will Have Fouling Coverage Less Than or Equal to (With 95% Confidence); and (B) the Standard Deviation of Percent Coverage (About the Mean) That These Samples Will Have As a Function of Increasing Sample Size.

V. CONCLUSIONS AND RECOMMENDATIONS

A. DISCUSSION

The choice of an appropriate sampling strategy for the study of fouling organisms in Monterey Bay has been shown to be dependent upon the type of information desired.

By analyzing the width of the upper 95% confidence limit about the expected value of the mean percent fouling cover, estimates were made concerning the optimum number of plates to be deployed. This optimum number was found by determining that point where the addition of additional plates had a negligible effect upon the width of the confidence interval. In nearly all the cases simulated, the value for the optimum number of plates to deploy appeared to be approximately twenty.

A similar procedure was followed to estimate the optimum number of plates to deploy to minimize the standard deviation about the mean percent fouling cover that 90% of the plates would have. The optimum number of plates to deploy to satisfy this requirement was again estimated to be approximately twenty for most of the simulations. However, the results from several cases suggested that thirty plates would be a more appropriate sample size.

Based on these results, it is concluded that twenty plates is probably the minimum number of plates that should be deployed to obtain accurate estimates of the mean percent fouling cover of a group of plates for this locality. Thirty plates per group is probably a more appropriate number of plates to deploy to insure that accurate results are obtained for groups that display heightened variability in the individual plate fouling coverage estimates.

The experimental results showing the negligible effect of the addition of more plates on what one could say 90% of the plates would have fouling coverage less than or equal to (with 95% confidence) lead to the conclusion that the inherent variability of fouling populations is significantly greater than previous studies indicated. This means that while twenty to thirty plates are probably sufficient to resolve ambiguities concerning the mean percent fouling cover, this number is clearly insufficient to ascertain with any high degree of confidence the amount of variability of a large segment of the population. For example, if it is desired to ascertain with 95% confidence the variability of 90% of the fouling population, it must be understood that this will require the committment of substantial resources to the study.

In addition to the development of an appropriate fouling community sampling strategy for Monterey Bay, more far reaching conclusions can be drawn concerning the applicability of the procedures used in this thesis to other locations. Since the computer modelling procedure used in this study dealt with the extension of the experimentally observed variability and contained no site-specific parameters, it is believed that the procedure can be directly applied to the study of fouling community variability at any desired geographical location. This means that the computer programs developed for this study can be coupled with archived fouling coverage data from any site, depth, season, and so on to provide information on how best to sample the fouling populations.

The final conclusion drawn from this study is that the bootstrap method of computer intensive statistical analysis has profound implications in the study of other biological problems. It is believed that this method could be applied to a wide range of other data intensive biological areas including fisheries management, larval settlement studies, the pelagic distributions of plankton and nekton, and growth rate studies to name just a few.

B. RECOMMENDATIONS FOR FURTHER RESEARCH

The model developed in this thesis should be applied to a number of other geographical locations to determine the

differences in fouling variability in terms of latitude, longitude, depth, environmental stresses, and a host of other biological forcing functions. Since the computer program already exits and the only required inputs are archived fouling coverage data, this should present no insurmountable difficulties.

Once such studies have been completed, the development of empirically derived sampling strategies for any location could be attempted.

Finally, the use of the bootstrap or other computer intensive statistical techniques should be vigorously investigated in other areas of biological interest. It is believed that the use of these techniques might well provide answers to a wide range of biological problems that have so far proved intractable.

APPENDIX A

MICRON 22 ORGANO-METALLIC
POLYMER ANTIFOULING PAINT

INGREDIENTS	PERCENT BY WEIGHT
Active:	
Bis (tributyltin) Oxide	11.7
Cuprous Thiocyanate	17.2
Inert:	71.1
	100

Elemental Tin 4.4%

Elemental Copper 8.9%

Paint contains 1.1 lbs of Bis(tributyltin) oxide per gallon and 1.6 lbs of Cuprous Thiocyanate per gallon.

Source: Product infromation breakdown on label

APPENDIX B

NAVY STANDARD FORMULA 121
RED VINYL ANTIFOULING PAINT

INGREDIENTS	PERCENT BY WEIGHT
Cuprous Thiocyanate	70.3
Rosin	10.5
Vinyl Resin	2.7
Tricreysl Phosphate	2.4
Methyl Isobutyl Ketone	8.1
Xylene	5.6
Antisettling Agent	.4

Source: Department of the Navy Specification MIL-P-15931C, Paint, Antifouling,Vinyl(Formula Numbers 121 and 129)

APPENDIX C

NAVY STANDARD FORMULA 170 BLACK
CAMOFLAGE ANTIFOULING PAINT

INGREDIENTS	PERCENT BY WEIGHT
Vinyl Resin	17.5
Bis (tributyltin) oxide	4.2
Tributyltin Fluoride	18.1
Carbon Black	2.1
Titanium Dioxide	.8
Ethylene Glycolmonoethyl Ether Acetate	3.0
Normal Propanol	11.1
Normal Butyl Acetate	43.2

Source: Department of the Navy Military Specification DOD-P-24588, Paint, Antifouling, Vinyl, Camoflage (Formula numbers 170, 171, 172, and 173), 2 May 1979.

APPENDIX D

ZYNOLYTE EPOXY RUST MATE PAINT

INGREDIENTS	PERCENT BY WEIGHT
Non-Volatile (58.4% of total)	
Pigments	43.4
Vehicle Epoxy and Menhaden Alkyd Resins	56.6
	100.
Volatile (41.6% of total)	
Exempt Mineral Spirits	98.0
Aromatic Hydrocarbons	2.0
	100.

Source: Product ingredient breakdown on label

APPENDIX E

A DISCUSSION OF THE METHOD OF MAXIMUM LINELIHOOD AND ITS INCORPORATION INTO A MODEL FOR FOULING COVER

A. GENERAL

The following model was used to describe the properties of the proportion of a plate that was fouled. Each plate (t) has a random proportion of fouling, P_t. Given P_t the number of the 100 censused sites that are fouled, (S_t), on the tth plate has a binomial distribution:

$$P\{S_t = k\} = \binom{100}{k} P_t^{\,k} (1 - P_t)^{100-k} \qquad (1)$$

for k = 0,1,2.,100 independent of the other plates. The proportion of fouling, P_t, is assumed to have the form

$$P_t = \frac{e^{\epsilon_t}}{(1 + e^{\epsilon_t})} \qquad (2)$$

where ϵ_t has a normal distribution with mean μ and variance σ^2 independent of the other plates censused at the same time. The mean μ and variance σ^2 will in general be a function of the amount of time the plate is submerged though the many variables involved in population dynamics will keep the function from being linear.

Assume N plates are inspected at a time with the result that S_t of the censused sites are fouled on the tth plate.

The likelihood function for the model is:

$$L = \prod_{t=1}^{N} \phi\{\epsilon_t;\ \mu,\sigma^2\} \binom{100}{k} P_t^{S_t} (1 - P_t)^{100-S_t} \qquad (3)$$

where

$$\phi\{\epsilon_t;\mu,\sigma^2\} = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{\epsilon_t-\mu}{\sigma})^2} \quad \text{for } -\infty \leq \epsilon_t \leq \infty \qquad (4)$$

Note that L is the probability of observing $S_1, \ldots, S_N$ fouled sites on each of the plates.

The method of maximum likelihood is to find those values of μ , σ , and ϵ_t , for t = 1,2,...N which maximize L; that is, those values which maximize the probability of observing the outcome. These values also maximize the log likelihood function, ln L, where

$$\mathcal{L} = \ell n\ L = \sum_{t=1}^{N} \{-\frac{(\epsilon_t - \mu)^2}{2\sigma^2} + S_t \ell n P_t - \ell n\sigma + (100-S_t)\ell n(1-P_t)\} \qquad (5)$$

[+ constant]

To find the maximum of $\mathcal{L}$ the partial derivatives of $\mathcal{L}$ with respect to μ , σ and ϵ_t are set equal to zero. This results in the equations:

$$\frac{\partial \mathcal{L}}{\partial \mu} = \sum_{t=1}^{N} -\frac{(\epsilon_t - \mu)}{\sigma^2} = 0$$

solving for μ results in:

$$\hat{\mu} = \frac{1}{N} \sum_{t=1}^{N} \epsilon_t \quad ; \qquad (6)$$

similarly

$$\frac{\partial \mathcal{L}}{\partial \sigma} = \sum_{t=1}^{N} \{\frac{(\varepsilon_t - \mu)^2}{\sigma^3} - \frac{1}{\sigma}\} = 0 \quad ;$$

solving for σ^2 results in

$$\hat{\sigma}^2 = \frac{1}{N} \sum_{t=1}^{N} (\varepsilon_t - \hat{\mu})^2 \tag{7}$$

and

$$\frac{\partial \mathcal{L}}{\partial \varepsilon_t} = - \frac{(\varepsilon_t - \mu)}{\sigma^2} + \frac{S_t}{P_t} \frac{\partial}{\partial \varepsilon_t} P_t + (100 - S_t) \frac{1}{(1-P_t)} [- \frac{\partial P_t}{\partial \varepsilon_t}] = 0 \tag{8}$$

where

$$\frac{\partial P_t}{\partial \varepsilon_t} = \frac{\partial}{\partial \varepsilon_t} [\frac{e^{\varepsilon_t}}{1+e^{\varepsilon_t}}] = \frac{e^{\varepsilon_t}}{(1+e^{\varepsilon_t})^2} = P_t(1-P_t)$$

Equation (8) simplifies to

$$\varepsilon_t + \frac{e^{\varepsilon_t}}{1+e^{\varepsilon_t}} (100\ \sigma^2) - \mu - S_t\sigma^2 = 0 \tag{9}$$

B. SOLUTION OF THE LOG LIKELIHOOD EQUATION

A recursive method was used to solve the system of equations (6),(7), and (9). For each month, the 100 sites were censused on each of the four untreated plates and S_t the number of fouled sites was determined. Initial values for $\hat{P}_t$, $\hat{\varepsilon}_t$, $\hat{\mu}$, and $\hat{\sigma}^2$ were determined as follows

$$\hat{P}_t(0) = S_t/100 \qquad (=\text{PNOT})$$

$$\hat{\varepsilon}_t(0) = \ell n[\hat{P}_t(0)/(1-\hat{P}_t(0))] \qquad (=\text{EPSNOT})$$

$$\hat{\mu}(0) = \frac{1}{4} \sum_{t=1}^{4} \hat{\varepsilon}_t(0) \qquad (=\text{MUNOT})$$

$$\hat{\sigma}(0) = \frac{1}{4} \sum_{t=1}^{4} (\hat{\varepsilon}_t(0) - \hat{\mu}(0))^2 \qquad (=\text{SIGNOT})$$

The values $\hat{\mu}(0)$ and $\hat{\sigma}^2(0)$ were then used in equation (9) and the equation solved for $\hat{\epsilon}_t(1)$, for t = 1,2,3,4. Since equation (9) is transcental, Newton's method was used to solve for $\hat{\epsilon}_t(1)$. Newton's method is an iterative procedure in which the (n+1)st member of the iterative sequence (X_n) is:

$$X_{n+1} = X_n + \frac{f(X_n)}{f'(X_n)}$$

where in our case

$$f(X_n) = X_n + \frac{e^{X_n}}{1+e^{X_n}} (100\ \hat{\sigma}^2(0)) - \hat{\mu}(0) - S_t\hat{\sigma}^2(0)$$

and

$$f'(X_n) = 1 + [e^{X_n}/(1+e^{X_n})^2]\ 100\ \hat{\sigma}^2(0)$$

Equation (10) was iterated until $|X_{n+1} - X_n| < 1 \times 10^{-6}$
The $\hat{\epsilon}_t(1)$, t = 1,2,3,4 were then used to compute iterated values of $\hat{\mu}$, $\hat{\sigma}$, and $\hat{P}_t$ as follows:

$$\hat{P}_t(1) = e^{\hat{\varepsilon}_t(1)}/(1+e^{\hat{\varepsilon}_t(1)})$$

$$\hat{\mu}(1) = \frac{1}{4} \sum_{t=1}^{4} \hat{\varepsilon}_t(1)$$

$$\hat{\sigma}^2(1) = \frac{1}{4} \sum_{t=1}^{4} (\hat{\varepsilon}_t(1) - \hat{\mu}(1))^2$$

and equation (9) was then solved with $\hat{\mu} = \hat{\mu}(1)$ and $\hat{\sigma} = \hat{\sigma}(1)$ for $\hat{\epsilon}_t(2)$, t = 1,2,3,4. The iterative procedure was continued until the achievement of a tolerance value of (1×10^{-6}) calculated by $|\hat{\mu}(k) - \hat{\mu}(k+1)|$ indicated the system of equations had converged. The final iterated values for $\hat{P}_t$ (=PNEW), $\hat{\epsilon}_t$ (=EPSNEW), $\hat{\sigma}^2$ (=SIGNEW), and $\hat{\mu}$ (=MUNEW) were the values that maximized the likelihood function for the model. SIGNEW ($\hat{\sigma}$) and MUNEW ($\hat{\mu}$) were then used as initial input values in the bootstrap simulations of the experiment (Appendix F).

APPENDIX F

BOOTSTRAP COMPUTER SIMULATIONS

A. AN EXPLANATION OF THE BOOTSTRAP METHOD OF COMPUTER SIMULATIONS OF RANDOM PROCESSES AND ITS INCORPORATION INTO AN ASSUMED STOCHASTIC MODEL FOR FOULING COVERAGE

1. Discussion

The common statistical tools utilized in the study of biofouling have as their basis the simplifying assumption that the data collected from the analysis of such communities can be described by a normal or Gaussian distribution. That is to say, it is assumed that fluctuations in the values of some experimentally observed parameter are scattered symmetrically about the true value of the parameter. It is further assumed that the larger the difference between the the true value and the observed value of the parameter, the less likely it is that the value will be observed experimentally (Diaconis and Efron,1983). Many years of experience using these assumptions have shown that even if the dataare only approximately or "pseudo" normal, the Gaussian theory still works quite well. If however, the data do not satisfy the requirements for the assumption of normality or,if the sample size is such that the various tests used to check for normality can only give ambiguous results, it is clear

that the results of statistical techniques based on the assumption of normality will be unreliable.

Recent developments in the use of computer-intensive techniques for statistical analysis, particularly the invention of the bootstrap technique (Efron,1977), have enabled the computation of various statistical parameters without the necessity of assuming a Gaussian distribution. This technique has also enabled the computation of those statistics which do not have a simple analytical formula. Prior to the advent of large main frame computers, the difficulty of finding numerical solutions to non-linear problems forced statistical methods to concentrate on those statistical models and procedures for which analytical results could be obtained. These models and procedures did give useful large sample size results for the common statistics such as the mean and variance of a population. However, they ignored other important statistical questions that did not have analytical formulas; such as, the degree of variability in an estimate due to the sample being of finite size or the confidence limits about estimates from a finite sample (Diaconis and Efron,1983). In general terms, the bootstrap method consists of coupling a probabilistic model with the data gathered experimentally from one sample of size N to generate a large number of simulated samples of size N. These simulated samples are then analyzed to

determine the variability of estimates of the true values of the statistics of the population.

2. Procedure

The model described in Appendix E was used in a simulation study of the variability of the estimate of the fraction of plate coverage. The model was used to simulate 200 groups of fixed numbers of plates. The inputs into the simulation were the values for the maximum likelihood estimates of the mean (MUNEW $\hat{\mu}$) and variance (SIGNEW $\hat{\sigma}^2$) for the monthly epsilon ($\hat{\epsilon}_t$) values determined from the analysis of the fouling cover on the four untreated plates.

The stochastic model used in this case assumed a normal distribution for the epsilon (ϵ_t) values (that is $\epsilon_t \sim N(\hat{\mu}, \hat{\sigma}^2)$). Using available computer software, the required number of normally distributed random numbers with mean equal to zero and variance equal to unity were generated for 200 groups of the following number of plates:

Number of Simulated Plates per Group	Required Number(I) of Random Numbers
2	I = 400
4	I = 800
5	I = 1000
10	I = 2000

Number of Simulated Plates per Group	Required Number(I) of Random Numbers
15	I = 3000
20	I = 4000
30	I = 6000
40	I = 8000
80	I =16000

Each of the (I) computer generated random numbers from the standard normal distribution were then transformed into random numbers(n_i) with a normal distribution with mean $\hat{\mu}$ and standard deviation $\hat{\sigma}$ by multiplying each i^{th}variable by $\hat{\sigma}$ (the square root of $\hat{\sigma}$) and adding $\hat{\mu}$. The random number n_i was transformed to give PINIT(I) defined as:

PINIT(I) = $e^{n_i}/(1 + e^{n_i})$, the average proportion of the i^{th} plate fouled.

The initial simulated percent cover for the i^{th} plate was determined by calculating a random number having the properties of a binomial distribution with probability (P) equal to PINIT(I) and N equal to 100. The resulting variable was termed PNOT(I). The variables EPSNOT(I),MUNOT, SIGNOT, PNEW(I),EPSNEW(I), MUNEW, and SIGNEW were then calculated using the method of maximum likelihood described in Appendix E.

APPENDIX G

TABULATED MONTHLY PERCENT FOULING COVERAGE VALUES
FOR THE NON-TOXIC CONTROL SURFACES

MONTH	PLATE #	FRACTIONAL COVERAGE
	1	.50
2	2	.50
	3	.24
	4	.54

MONTH	PLATE #	FRACTIONAL COVERAGE
	1	.33
3	2	.49
	3	.69
	4	.46

MONTH	PLATE #	FRACTIONAL COVERAGE
	1	.73
4	2	.02
	3	.07
	4	.84

MONTH	PLATE #	FRACTIONAL COVERAGE
	1	.13
5	2	.96
	3	.61
	4	.81

MONTH	PLATE #	FRACTIONAL COVERAGE
	1	.54
6	2	.46
	3	.41
	4	.06

MONTH	PLATE #	FRACTIONAL COVERAGE
	1	.54
	2	.47
7	3	.62
	4	.31

MONTH	PLATE #	FRACTIONAL COVERAGE
8	1	.48
	2	.88
	3	.69
	4	.97

MONTH	PLATE #	FRACTIONAL COVERAGE
9	1	.91
	2	.49
	3	.92
	4	.35

MONTH	PLATE #	FRACTIONAL COVERAGE
10	1	.70
	2	.56
	3	.29
	4	.97

MONTH	PLATE #	FRACTIONAL COVERAGE
11	1	.83
	2	.95
	3	.64
	4	.98

APPENDIX H

LIST OF THE SESSILE SPECIES IDENTIFIED BY THE RANDOM POINT CENSUS AND THE MONTHS THEY WERE PRESENT ON THE NON-TOXIC CONTROL SURFACES

	MONTH NUMBER									
ORGANISMS	2	3	4	5	6	7	8	9	10	11
Protozoa:										
Folliculina spp.	X	X	X		X	X		X		
Ephelota gemmipara	X									
Coelentrata:										
Obelia spp.	X	X	X	X	X	X	X	X	X	X
Hydractinia spp.					X		X			
Anthopleura spp.										X
Ectoprocta:(Bryozoans)										
Bugula neretina			X			X	X	X	X	X
Bugula californica	X	X	X				X			
Watersipora cucullata	X	X	X	X	X	X	X	X	X	X
Hippothoa hyalina	X	X	X	X	X	X	X	X	X	X
Celloporaria brunnea	X	X	X	X	X	X	X	X	X	X
Cryptosula pallianasa			X	X	X		X	X	X	X
Schizoporella unicornis			X	X	X	X	X	X	X	X
Microporella ciliata			X	X	X		X			
Microporella californica									X	X
Membranipora membranacea									X	X
Membranipora serilamella					X				X	
Unknown bryozoan #1									X	
Unknown bryozoan #2										X
Annelida:										
Circeis armoricana	X	X	X	X	X	X	X	X	X	X
Janua nipponica				X			X			
Pileolaria potswaldi				X			X			
Protolaeospira exima	X									
Serpula vermicularis							X	X		X
Anatides groenlandica	X				X					
Arthropoda:										
Balanus crenatus			X					X		
Megabalanus californicus					X		X	X	X	X
Amphipod (unknown)						X	X			
Mollusca:										
Mytilus edulis										X

ORGANISMS	MONTH NUMBER 2	3	4	5	6	7	8	9	10	11
Echinodermata:										
Strogylocentrotus spp.										X
Chordata:										
Ascidia ceretodes					X		X	X	X	
Styela truncata					X					
Pyura haustor										X

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