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GUIDELINES:

BIOLOGICAL SURVEYS AT PROPOSED HEAT DISCHARGE SITES

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GUIDELINES: BIOLOGICAL SURVEYS AT PROPOSED HEAT DISCHARGE SITES

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PREFACE

The key words in the title of these Guidelines are "proposed --- sites." The modern goal of environmental quality control on many fronts is pollution prevention and environmental protection. The horizons are moving out past simple abatement of existing pollution and recovery from damages. Heat pollution is not unique in this respect.

In keeping pace with the changing times, the scientist and engineer are faced with different and more complex problems. It had been a comparatively simple matter to survey an existing source of heat pollution and to document its effects by measuring temperature gradients of the receiving water and collecting biologic data within and without the zones of temperature increase. In surveying a proposed discharge site, the investigators are faced with two new challenges. First, they must design a sampling program without absolute knowledge of how the heat will behave, collect data to assay the biologic resource in jeopardy, and assure that the data are adequate for defensible pre- and post-operational comparison in the event that some heat discharge does occur. Second, their pre-operational data and their form of presentation must be sufficiently conclusive to influence and support very important, and probably costly, management decisions.

The authors present here general survey procedures that have a reasonably high probability of success anywhere. Deviations from the suggested procedures should be made only on the basis of special knowledge of the peculiarities of a specific site and for the purpose of raising the probability of success.

ABSTRACT

A quantitative approach is presented for the biological portion of thermal discharge siting surveys and discharge monitoring. Three types of studies are covered: Type I is a very general study of the aquatic system and the pertinent literature; Type II is a comprehensive pre-operational study designed to supply data for management decisions on power plant siting and data to serve as baseline for possible future comparison; and Type III is a post-operational continuation of Type II to detect possible effects if a thermal discharge to a natural water body is allowed.

Two methods are recommended for location of sampling stations by use of a grid system based on planned outfall design. Sample collection and handling methods and frequency of sampling are suggested for fish, macroinvertebrates, plankton, periphyton and aquatic macrophytes.

Methods of data handling recommended include diversity and redundancy indices and a combination of the two into one value for a test for dispersion. A scale of importance is suggested for organisms of special value in either an economic or ecologic sense. For statistical analysis of the data, four appropriate methods are recommended and sample problems are provided to illustrate data handling and conclusions to be drawn from the tests.

Key Words: Thermal pollution, siting surveys, biological sampling, data analysis, thermal power plants, ecological studies.

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INTRODUCTION

This publication provides a plan for prediction and detection of biological effects of thermal additions to the aquatic environment. A quantitative approach is presented for pre-installation sampling at sites of potential thermal discharges and for monitoring of biological effects attributed to these discharges.

Waste heat is a significant pollutant in the United States. Heat added to natural bodies of water in this country comes from many sources including industrial processes, air-conditioners, and irrigation, but the largest amount comes from the electric power generating industry. In 1964, almost one-half of the water used in the United States was for cooling and condensing by the power and manufacturing industry (1). Electric power plants, which use stream, reservoir and lake waters to cool condensers, used about 80 percent of this cooling water. Therefore, the projected growth of electric power production provides a good index of thermal pollution potential for the near future. Power generation has approximately doubled each ten years during this century and estimates of future demands indicate a shortening of the time span for similar increases (1, 2). Heat rejection from nuclear and fossil power plants is expected to increase almost nine-fold by the year 2000 (3) and there will be an accompanying rise in waste heat output from manufacturing industries. The net effect will be a great increase in the amount of waste heat rejected to the environment.

This additional waste heat must be managed in a manner that will maintain or enhance the physical, chemical, and biological nature of water resources. The environment must be protected by judicious selection of industrial sites for development, by incorporation of waste heat treatment into plant design and operation, or by regulation of discharge of waste heat to conform with water quality standards. In all cases, the potential ecological effect of thermal discharges must be considered.

To predict the effects of thermal discharges on the aquatic environment and on our use of this public resource, we must first be able to assess the nature of the environment as it is at present. To do this, we must have adequate and reliable techniques for sampling and evaluating the aquatic biota in the areas of potential effect.

A biological survey conducted at a potential site of thermal discharge should:

1. Establish the nature and value of the resource in jeopardy.
2. Provide a basis for projection of consequences of thermal discharges and for determination of controls to prevent detrimental effects.
3. Provide a baseline which can be used for future comparison.

If a thermal discharge is allowed, a monitoring program should provide data for detection of possible changes in the biota.

Minimum amount of time and expense is required to accomplish the task of evaluation if the essential data are obtained without wasted effort and the data are of maximum utility. These points are considered basic to the sampling program described in this paper.

The emphasis in this paper is upon the biota, hence, results should be interpreted by an aquatic ecologist. However, the complexity of the problem requires a multidisciplinary approach and the advice of competent chemists, engineers, and statisticians must be available if needed.

PLANNING THE SURVEY

Careful planning of the survey is necessary to assure (1) that adequate information is obtained for a true characterization of the aquatic community and (2) that valuable time and energy are not spent collecting useless data.

Survey Types

Site surveys fall into three broad categories based upon the amount of detail required at the time. The three types of surveys fit into a natural sequence.

The first type is a very general survey of the area and of the pertinent literature. This survey could be conducted in a very short time with a minimum amount of manpower and expense. It provides a general idea of the kinds of organisms present and a rough idea of their relative numbers. In some cases, this information might be sufficient in itself to determine whether a proposed thermal power plant should be installed and if once-through cooling should be allowed. As an example, if the preliminary survey shows that the proposed site is an important spawning area for cold-water fish, a heated effluent should not be allowed in this area. When the preliminary survey is not conclusive, it can be used in planning the second type of survey.

The second type is a much more detailed survey requiring more manpower and money. This survey should provide an appraisal of numbers and kinds of organisms present as well as data on important chemical and physical characteristics of the environment. This survey should furnish regulatory agencies with the information needed for the final decision as to whether a thermal discharge should be allowed at that proposed site, and if so, what controls should be imposed.

The third survey type is a continuation of the second. If the power plant or other potential source of thermal pollution is installed, the receiving body of water must be monitored to detect any changes and to plan remedial action if such changes are detrimental to the resource. Because of the variability from year to year in biota and meteorological conditions, the combination of the second and third type surveys should include at least three years pre-operational data and at least three years post-operational data. This may vary somewhat, depending on the nature of the area and the degree of annual variability encountered. The plans for the second and third phases should be the same since both sets of data must be complete enough to give a detailed and reliable picture of the numbers and kinds of biota present. In these phases, adequate statistical planning is essential to ensure that post-operational data can be compared with pre-operational data to detect significant changes.

Organisms to be Sampled

Since the basic purpose of these surveys is to aid in protection of the public resources, it is logical that most of the organisms studied will be those which are of special importance to the public. These include such commercial species as salmon, shellfish, etc., and fish such as trout and bass which are important for recreational purposes. Essential organisms in the food web of these species are also important. Nuisance forms such as blue-green algae may be very significant because their presence decreases the recreational value of a body of water.

Expected changes in fish populations will vary with species and with the chemical and physical characteristics of the receiving water. In an extreme case, the fish may be killed by the hot water, but the effects are nearly always more subtle. Fish may be driven out of a heated area at high temperatures, but attracted at lower temperatures. Not all fish will be affected in the same way or to the same degree, so there may be changes in species composition. Fish passageways or spawning areas may be blocked to eliminate a species in some areas, but overall productivity may be increased in others. The range of possibilities is great and the sampling problems will be unique to each site.

Benthic macroinvertebrates are sampled for three principal reasons:

1. They are important members of the food web and their well-being is reflected in the well-being of the higher forms such as fish.
2. Many invertebrates, such as the marine shellfish, are important for commercial and recreational use.
3. Because of their relative immobility, they are especially good indicators of pollution at the bottom of the water column.

A feature of their use as indicators of pollution is the fact that they do not reflect average levels of pollution, but are more likely to reflect the extremes. The invertebrates cannot avoid pollutants and many are slow to repopulate an area, so detrimental effects are not easily erased. Consequently, the structure of the community may be determined during those months in which temperatures are highest.

The use of benthic species for detection of thermal effects is limited by the unique property of the heated effluent water. The effluent may be dispersed to float on the underlying receiving water without ever coming in contact with the benthos. Still, benthic sampling cannot be eliminated because a floating heated layer may promote oxygen problems and may kill emerging aquatic insects or planktonic stages of marine invertebrates.

Zooplankton are essential in most aquatic systems, but due to the high variability in numbers and species composition, it is very difficult to arrive at valid conclusions using zooplankton data unless samples are taken in greater numbers and with more frequency than will usually be practical in the type survey being proposed here. Also, in a body of flowing water, the zooplankton at any spot are largely the product of upstream conditions and not of conditions at the point of sampling. Because of these problems, pre-operational zooplankton data at potential sites of heat discharges are of dubious worth and only limited and specialized plankton sampling is recommended.

In some areas, it will be necessary to sample for the immature planktonic forms of fish and shellfish. In estuaries or the ocean, the planktonic eggs and larvae of many species may be killed by passage through the condenser system. Thus, the invertebrate community could be severely harmed without the hot water layer ever touching the bottom where the adults are found. In fresh water, the same thing may happen to young fish. The presence of such planktonic forms must be determined as part of the Phase I and II siting surveys.

Phytoplankton are considered primarily because of their potential for nuisance. An increase in water temperature due to waste heat may cause an increase in productivity of phytoplankton and also cause a species shift to less desirable forms. Cairns (4) found that while there is significant species overlap, it is evident that a rise in temperature is generally accompanied by a gradual shift in algal species from the diatoms to the greens and finally to the blue-greens at the highest temperatures. In terms of desirable productivity for fish and other aquatic life, the shift would be from more to less desirable forms. Eutrophication of lakes and reservoirs is already becoming a very serious problem in the United States and addition of waste heat could contribute to this problem.

Periphyton samples are taken for an estimate of primary productivity. Since periphyton includes the attached algae, an estimation of algal growth is obtained with less problem of natural variability than is encountered in sampling phytoplankton.

Aquatic macrophytes provide both food and shelter for fish and fish-food organisms, and they may provide important feeding and nesting sites for shore birds and waterfowl. Perhaps even more importantly, aquatic macrophytes may become too plentiful and create a nuisance by clogging water intakes and by choking canals and bays to such an extent that boating is impossible and flow for irrigation or other uses is severely restricted.

An increase in temperature may cause a shift in species composition of aquatic plants or may cause accelerated growth of the plants present. Anderson (5) stated that at Chalk Point power plant on Chesapeake Bay, a species of *Ruppia* which grew profusely in the area and was used as food for migrating waterfowl was replaced by a species of *Potamogeton* within two years after the power plant began to discharge heated water into the bay. On reservoirs administered by the Tennessee Valley Authority, there is already a serious problem due to clogging of waterways by large growths of *Myriophyllum*. *Ceratophyllum* and *Elodea* have produced similar problems in other places. These problems have not all been caused specifically by rises in temperature. However, a temperature rise might promote the problem in areas where additional heat could increase the length of the growing season. It is especially important to prevent the growth of such plants because eradication becomes a long and costly procedure once larger areas are infested.

Measurement of selected chemical and physical parameters should be included in Type II and III surveys. Without such data, it will be difficult to determine conclusively whether biologic effects result from temperature alone, other pollutants associated with the discharge, or other causes.

Location of Sampling Stations

To ensure that the sampling effort will result in data which can be treated statistically, the sampling areas and times must be determined in advance. Sample stations should be located on a grid system designed to cover the heat-affected portion of the water body and a portion of the surrounding area. The heat-affected areas will not be known precisely prior to installation of the plant, so the location of the sample grid will be based upon engineering predictions of movement and behavior of the heated effluent.

The two sampling grids presented in this section are for example and will not fit every situation. The grids should be modified to fit the morphology and hydrology of the site, but the basic idea of sampling within and outside of the proposed heated area can still be followed.

Rivers

In a river, samples should be taken on at least four transects (See Figure 1) located in relation to the plume area. References (6, 7, 8, 9, 10) may be consulted for assistance in predicting the extent of the thermal plume.

The first transect should be close to the effluent on the upstream side, but out of the influence of the heated discharge. The second transect should be close to the effluent on the downstream side and within the heated plume. The third transect should be placed at approximately that point where the surface temperature in the center of the plume has dropped to the point halfway between ambient river temperature upstream from the effluent and temperature of the effluent ($0.5\Delta T$). The fourth transect should be located at the point where a rise in river

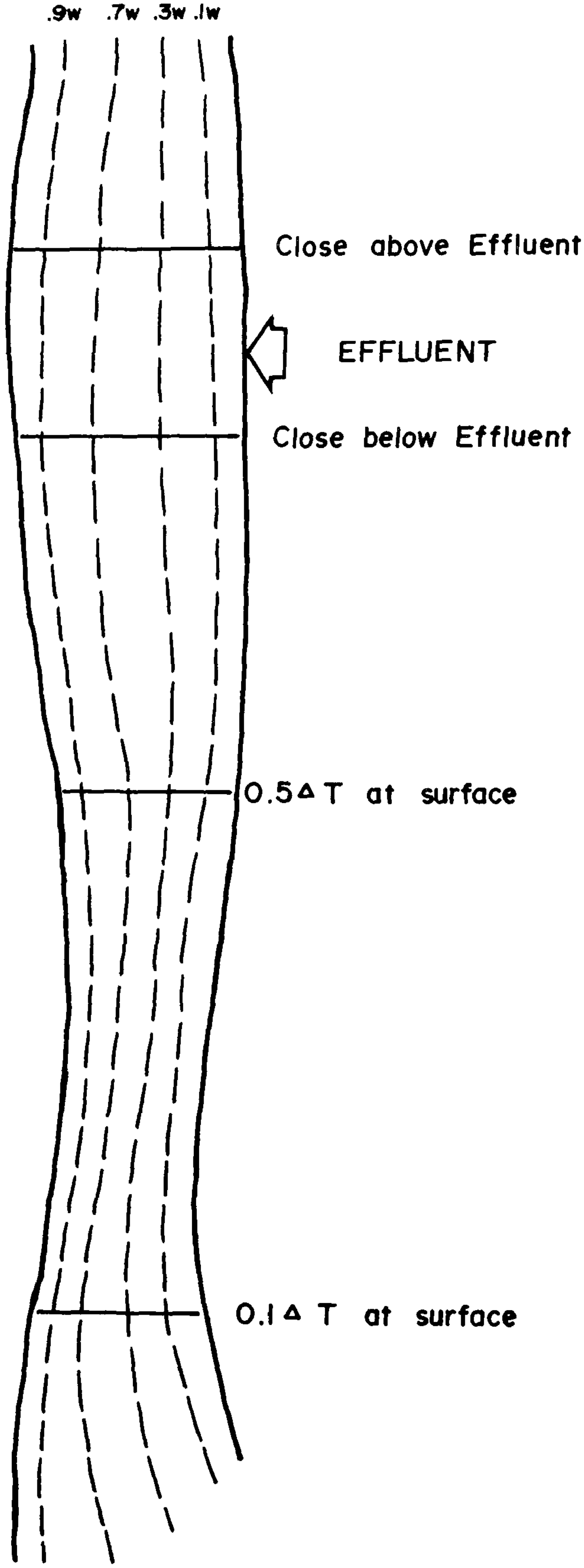


FIGURE 1 ····· TRANSECT GRID FOR RIVER SAMPLING temperature is barely detectable ($0.1\Delta T$). Location of the lower two transects will be determined for that time of year when the river within the plume area is expected to be at maximum temperature and will be fixed thereafter.

For all organisms except fish, four sampling stations will be established on each transect. These stations will be located at 10, 30, 70, and 90 percent of the width at that time of year for which the downstream reach of the plume is calculated. If the nearshore area is appreciably different from the center sections (more shallow, weedy, etc.), additional stations should be located between the banks and the 10 and 90 percent stations. In a river in which width fluctuates widely, it is important that the sampling stations be located where they will not be dry at low flow. The four or more stations on each transect should be permanently buoyed so that samples for each period can be taken at the same spot. Depths will be mentioned later for each type of sample.

For fish, the river should be divided along each transect into three equal widths consisting of one-third of the width from each bank and one-third in mid-channel. Samples will be taken in these areas in the general vicinity of the transect line since most fishing gear does not lend itself to precise placement on a line. Additional stations may be necessary to include special migratory routes or other areas of fish concentration.

For a post-operational survey, the same stations will be sampled, but additional transect lines may be required to provide sufficient information to establish the boundaries of thermal effect.

Lakes, Oceans or Estuaries

In lakes, reservoirs, or estuaries where the flow is of sufficient velocity and persistence to resemble a river, the river transect plan may be applied. But in most large lakes or at ocean sites, a different sampling plan based upon distance from the outfall is warranted.

In these cases, a grid system should be established to include the area of expected temperature change and enough of the surrounding area to provide an unaffected zone for comparison.

A suggested method is to locate sampling stations on the intersections of concentric circles about the outfall and transect lines radiating from the outfall (See Figure 2).

The concentric circles should be placed to pass through the most distant points where surface temperature increases above ambient are predicted to be $0.9\Delta T$, $0.5\Delta T$ and $0.1\Delta T$ at that time of the year when the highest temperatures and greatest affected area are predicted. References (11, 12, 13, 14) may be consulted for assistance in predicting the extent of the thermal plume.

To set up the transect lines, determine the number of degrees of angle of open water in the outermost concentric circle. Run the transect lines from the point of effluent out through the points on

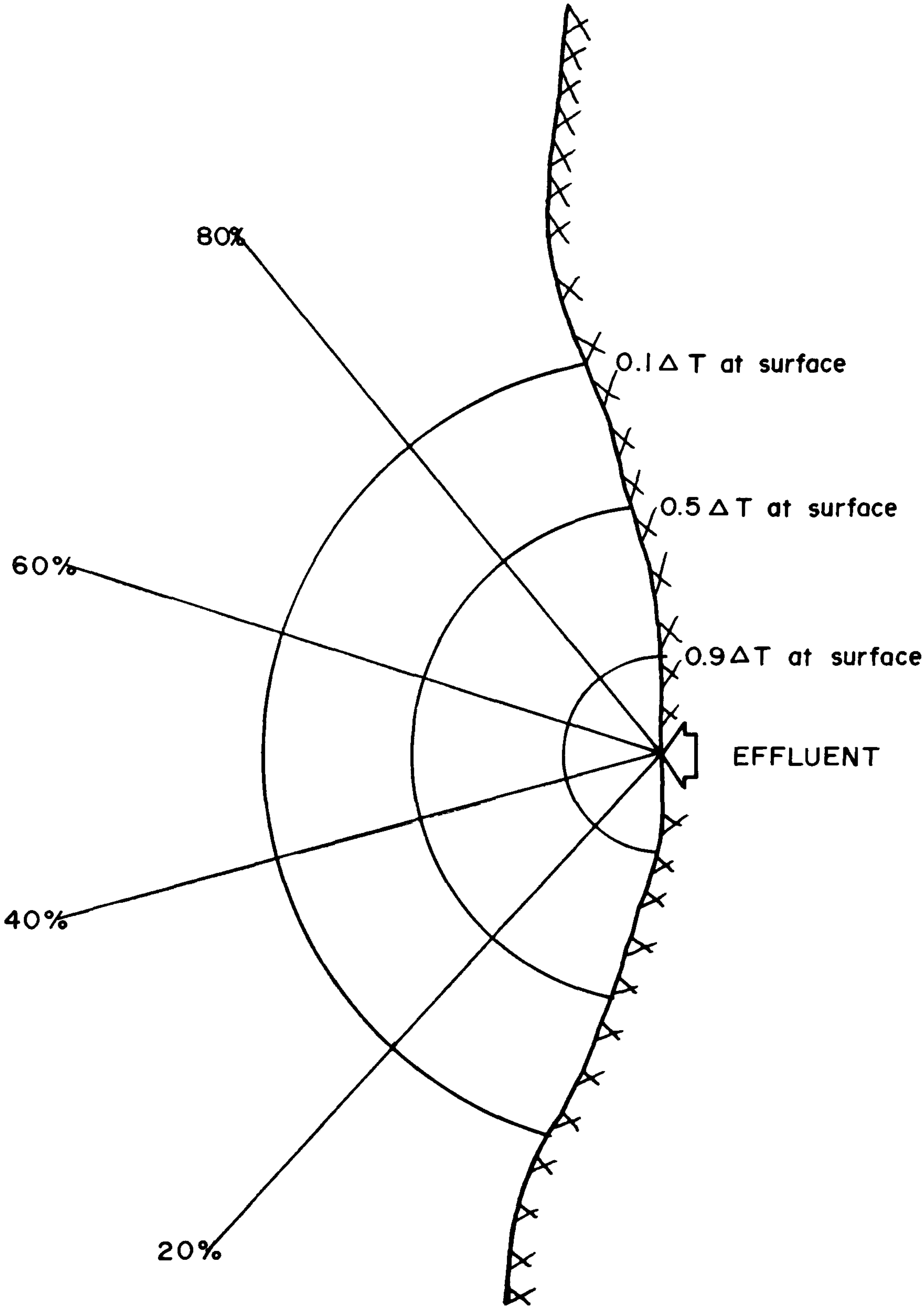


FIGURE 2 TRANSECT GRID FOR LAKE OR OCEAN SAMPLING

the circle at 20, 40, 60, and 80 percent of the angular distance from the shore on one side of the effluent to the shore on the other side. For example: if the shoreline runs north and south, the open water portion of the circle will be 180°. The points on the circle will be at 20, 40, 60, and 80 percent of 180° or at the 036, 072, 108, and 144 degree points in relation to north.

This suggested method for setting up the sampling grid may have to be changed to fit local conditions. More transects should be added if the plant is on a point where the open-water portion is so great as to put the transects too far apart and additional samples may be needed along the shoreline if the 20 and 80 percent radials are too far out to ensure sampling of shallow inshore areas.

Sampling Methods

Sampling methods are recommended herein for many kinds of organisms in different environments, but it is not expected that all types of sampling would be used on each survey. The preferable sampling methods differ with each individual case. In each survey, it will be desirable to sample benthos, fish, etc., but the methods of taking the samples are to be chosen by the biologist-in-charge to suit the situation.

Sampling methods are presented for organisms grouped by type or by habitat preference. The statistical treatment of the data discussed in sections to follow should be determined at the beginning of the survey to ensure that sampling and analysis methods are compatible.

Fish

Duplicate samples should be taken at each station to quantify the variability in sampling results. This may be done by sampling during two successive periods or by sampling in two spots at a station simultaneously. Fishing methods, once chosen, should remain constant, and the sampling series should be conducted at the same time each year.

Sampling should be conducted with sufficient frequency to detect seasonal migrations and fluctuations in the population. For example, anadromous species are in a river during two specific periods. The young fish drift downstream after they hatch and, later, the same fish move back upstream to spawn. Sampling times and methods must be planned to sample the young fish moving downstream and the adults coming back up. In this case, there would be just two general sampling periods per species. But there may be several anadromous species in one river so several sampling periods would be needed to catch every one. The same principle may be applied to bass or any other warmwater fish which moves into special areas of lakes or streams for spawning. Sampling for non-anadromous species should be conducted at least four times each year to coincide with the four seasons.

Each sampling series should provide the following: (1) identification of species present; (2) number of individuals of each species; (3) biomass of each species; and (4) scale samples, if suitable, for later use in age and growth studies. Numbers and weights may be converted to numbers or weights per catch effort if effort varies significantly from one series to another.

The following is a list of suggested sampling methods. The methods and gear must be tailored to each species at each site and a combination will often be required to sample adequately every important species of fish. The important thing is to obtain adequate reliable data by whichever means are most suitable.

1. Drag seine - Relatively nonselective for both species and size (very small fish may go through the mesh), but its use is generally limited to small rivers or embayments where fish may be trapped in small areas. Hoover (15) checked by mark and recapture and dynamiting on small trout streams and determined an efficiency of 70 to 100 percent. Gerking (16) found 88 percent efficiency in a warmwater stream in Indiana. In thermal studies, it would be most useful in backwaters or embayments or in small streams used for industrial discharge.

2. Gill nets and trammel nets - These are used in both streams and still water such as in lakes or estuaries. Their use is limited by current which tends to clog the net with floating debris; drifted nets are hampered by obstructions on the bottom. They are selective for species and size of fish, but can be adapted by changing mesh size. Cleary and Greenbank (17) found that they did not work well with deep-bodied fish such as the Centrarchids, but that they are quite efficient for many other fishes.

3. Traps - There are several types of trap nets so the best one must be chosen to fit the species and the morphology of the area. Hoop nets and wing nets are limited by current and movement of the fish; however, Cleary and Greenbank (17) state that workers in eastern Iowa and field men of the Upper Mississippi River Conservation Committee have found the trap net, despite its limitations, to be the best all-around method of taking qualitative and quantitative samples of fish in large streams. A mobile fish trap suspended between two outboard motor boats was used successfully to capture young salmon in Bellingham Bay, Washington (18).

4. Electric shockers - Their use has been primarily in small streams and lakes (19). Large streams and reservoirs would be very hard to sample this way, but the shocker may be used in special situations where fish are restricted to shallow areas.

5. Townets and trawls - These nets are useful for large reaches of open water such as estuaries, bays, reservoirs and the ocean where they have been used extensively for studies on Pacific salmon (18, 20). The depth of tow must be adjusted to the fish sought and morphology of the area.

6. Mark and recapture - This method entails release of captured marked fish and subsequent recapture by any one of the fishing methods already mentioned. A ratio is then set up for each species:

$$\frac{\text{Marked fish recaptured}}{\text{Total marked fish}} = \frac{\text{Total fish captured}}{\text{Total fish in the water body}},$$

to estimate total numbers of fish (20, 21). This method assumes no significant loss or recruitment to the population between capture series.

7. Electronic tracking - Tags which give off sonic or radio waves are useful for tracing migration patterns of anadromous fishes (22, 23) and may be very helpful in determining fish passageways or other key sampling areas.

These are not the only available methods and no one will work in all cases, but they are examples of successful methods being used for fisheries surveys.

Where a substantial commercial or recreational fishery exists, catch records by periods or by units of effort may furnish valuable data for estimations of the fishery. They are difficult to interpret as a measure of absolute numbers of fish present, but they do provide information on fluctuations in abundance. If the commercial or recreational fishery is especially important, the catch records may be as pertinent as records of total abundance of the species.

However, consideration must be made of the fact that commercial catch records often are influenced by factors other than abundance, such as fluctuating price of fish, local opportunity for other more lucrative jobs, or fishing techniques used.

The ideal study of the fishery would include estimates of all species of fish regardless of their commercial or forage value. However, if budget or other considerations limit the intensity of the study, it is more practical to work on a few species of fish such as those receiving heaviest angling pressure. In this case, specialized methods can be developed for the fish in question and such population characteristics as ratio of adults to subadults, rates of growth, etc., can be determined.

The information obtained must be used by regional ecologists to predict (or, in Type III, detect) heat-induced changes in the fish populations. This requires adequate temperature-tolerance data for the fish and adequate data are not now available for all species. In such cases, the workers will have to establish these tolerances at the time of the survey by means of bioassay experiments. The predictions of the ecologist can then be used to judge the suitability of the site for a power plant and the possible restrictions which must be imposed if the site is accepted.

Macroinvertebrates

Invertebrates should be sampled at those permanently buoyed locations mentioned in the section on location of sampling stations and all series should be taken at the same locations. Duplicate samples should be taken at each site, and the following information recorded from each sample: (1) identification of species (or genus if appropriate); (2) numbers of individuals per species; (3) biomass per species.

Since many of the invertebrates (aquatic insects, for example) have relatively short life cycles, the sampling program must be carried on throughout the year to detect seasonal changes. Invertebrate bottom samples should be taken a minimum of once each quarter. Artificial substrate samplers should be changed every six weeks unless growth is so heavy that a shorter period is indicated. In this case, they should still be retrieved at six-week intervals, but set out so as to be exposed for a shorter time.

Sampling devices must be suited to the substrate and the organisms to be sampled, but generally the choice will be made from a few methods or a combination of methods. Before methods are determined for the long-term Type II and III surveys, the preliminary, Type I, survey should be conducted. This quick survey with a variety of gear will enable the researchers to determine roughly the kinds of organisms and type of substrate to be sampled so gear can be chosen for the extended Type II and III surveys.

The following references will be useful for selection of sampling methods and gear:

Bottom samplers - 24

SCUBA sampling methods - 25, 26

Artificial substrates - 24, 27, 28, 29, 30

Sample handling is much the same no matter which type sampler is used. In any case, the invertebrates will be sorted, counted and identified, and weighed. See references (24, 29, 30) for sample handling information.

As in the case of fish, prediction of effect requires reliable temperature tolerance data for the forms present and this is not always available. Predictions may sometimes be made on the basis of data for related forms but for species of special importance bioassay data may have to be acquired at the site.

Plankton

Planktonic animals. Occurrence of those organisms normally known as zooplankton is so variable that the information gained will generally not be worth the expense in siting surveys. But, many of the commercially valuable invertebrates, such as clams and oysters, have planktonic stages which must be included in the survey. The presence of these forms can be inferred from the benthic samples of adults which have planktonic eggs or larvae, but actual distribution will have to be determined by sampling with plankton tow nets or other devices which filter a measured amount of water (18, 24).

Sampling should be conducted during the seasons when such planktonic forms are present in the system. In some cases, this may be only during a few months of the year. Samples should be taken on the grid system so the same area can be sampled each time, but additional samples may be chosen to include areas of concentration which the grid does not cover adequately.

Local currents and concentrations of planktonic forms should be taken into special consideration for design of intake systems to avoid harmful effects. If such devices are built, they must be monitored in the Type III survey and the effluent water from the condensers must be sampled to determine if the condenser temperatures are lethal to those organisms going through the intakes. If these temperatures are lethal, one can estimate the numbers of planktonic organisms killed by determination of flow rate of organisms through the condensers.

In sampling for the lethal effects of condenser passage, it is extremely important that the organisms be held long enough after passage to detect the effects. Organisms may appear well immediately after passage, but be dead a day later as a result of the temperature shock or other injuries. Exact timing will depend upon the organisms and on preliminary tests but it should be fixed after the study starts if results are to be comparable to detect possible changes in mortality due to changes in season, plant operation, etc.

Phytoplankton. In large open bodies of water, the phytoplankton are so variable in number and distribution that sampling usually does not produce data worth the time and effort expended. But, the planktonic component of the algae is of interest in enclosed bodies of water such as small lakes or estuaries where the entire life cycle of the organisms would be affected by the heated water. This portion may be sampled by standard plankton techniques using a net which strains a measured amount of water. Phytoplankton are then concentrated and estimates are made of percent blue-green algae, green algae and diatoms, ash-free dry weight and chlorophyll. This sampling should be conducted at the buoyed stations determined by the grid system at the same six-week intervals used for invertebrate substrates. See Reference 24 for details of sample collection and handling.

Data may be presented in graphic form and an analysis should be made as suggested in the statistics section to detect shifts in algal types. Special note should be made of potentially troublesome forms.

Periphyton

Periphyton should be sampled with periphyton substrates, such as the plexiglass plates used by Grzenda and Brehmer (31) or the glass slides used by Patrick, et al. (32), and recommended in FWPCA Methods for the Collection and Analysis of Freshwater Plankton, Periphyton, and Macroinvertebrate Samples (24). These artifical substrates are held in racks and submerged at a predetermined depth (24) at the buoyed sampling spots determined by the grid system used. Slides should be exposed for two- to four-week intervals (24) and samples should be taken at six-week intervals to coincide with macroinvertebrate substrate sampling.

Samples should be analyzed for percent diatoms, green and blue-green algae, chlorophyll and ash-free dry weight (24).

Aquatic Macrophytes

In determining suitability of the site for heat discharge or in monitoring effects, the main points to consider are: (1) distribution and abundance of present plant growth and (2) suitability of bottom type for extension of these plant beds should growing season be extended or conditions in some other way be made favorable for plant growth.

A preliminary requirement is that existing plant beds be mapped to show areas covered by each species. Such mapping is greatly facilitated by use of regular and infrared aerial photography accompanied by ground check to provide species identification (33). In this manner, the entire area likely to be affected by heated effluent can be mapped in a relatively short time without expenditure of a great deal of time or money. Special consideration should be given to water depth in the area. Distribution of most of the attached aquatic plants is limited by depth so there will be little danger of excessive growth of rooted aquatics in deep, steep-sided reservoirs or rivers. The critical regions are in lakes or rivers with extensive shallow littoral areas with bottoms suitable for attachment of aquatic plants. Morphology of the bottom should be determined as part of the general overall survey.

The aquatic plant survey should be made at least three times during the year if the potential for nuisance exists. It is of primary importance that the main survey be made during the middle part of the summer when aquatic plant growth is at its maximum. Comparison of pre- and post-operational data collected during spring and fall surveys will show whether or not plant growth or distribution is affected by lengthening of the growing season as a result of addition of the hot water.

Aquatic plant data can be presented as units of area covered or as percent of total area covered by each species. If percent is used, replication is not necessary and the data can be presented graphically or analyzed as suggested in the section on statistics.

Chemical and Physical Parameters

The following chemical and physical parameters should be included in a minimum sampling program:

1. Dissolved oxygen determinations should be made each season during periods of maximum temperature and lowest river flow, or at any other time of year when oxygen concentration may get low enough to be a potential problem. This must be done during the siting surveys and continued throughout the monitoring program until it is demonstrated that no problems have been created.

2. Surface to bottom temperature profiles should be determined at each grid station at six-week intervals when the benthic substrates

are collected and temperatures at the intake and outfall should be continuously monitored.

3. Residual Chlorine. If a discharge is allowed, this should be monitored upstream from the discharge and in the discharge area if chlorine is used as an algacide. Monitoring should be planned to coincide with the plant chlorination schedule so slugs of chlorine will be detected.

4. Dissolved copper, nickel and zinc. If a discharge is allowed, these metals should be monitored upstream from and within the discharge area until it is determined that there is no toxic metal leaching from any part of the power plant system.

5. Nitrogen series (dissolved, nitrite, nitrate, ammonia, Kjeldahl). These determinations must be made as part of the initial survey at the buoyed sample stations at six-week intervals to determine potential for increases in possible nuisance algae.

6. Phosphorus (total and ortho). Conduct the same as nitrogen.

7. pH, hardness and alkalinity. Conduct the same as nitrogen and phosphorus.

8. Pesticides should be monitored before and after installation if pesticide use is prevalent enough in the region to mask the effect of temperature.

9. Salinity should be monitored in the marine environment on the same schedule as nitrogen and phosphorus if the discharge

and intake are arranged so as to cause drastic changes in currents or in salinity of estuaries.

Samples should be taken one foot below the surface, at mid-depth and one foot above the bottom at each of the sample sites designated. (Temperature profile is taken at one-foot intervals at the same site.) Analysis should be made in accordance to FWPCA Methods for Chemical Analysis of Water and Wastes (34).

DESIGNING THE STATISTICAL MODEL

This section outlines a few statistical techniques to be used for evaluation of data collected by the recommended sampling methods. More detail on specific tests is included in the Appendix. Obviously, the materials and procedures presented herein do not cover all possible situations. Before an investigation is begun, the project officer and key staff should consult with a statistician to design the survey and subsequent analysis to account for factors peculiar to the environment being studied.

The statistical model should provide for the control of factors in the system which affect the response factor. Affecting factors are of two types: (1) those which can be fixed, such as transect location or season; and (2) those which, although not fixed, can be measured, such as velocity, dissolved oxygen, nutrients, etc. This second set of factors is examined to determine that they are not lethal or do not override the effect of temperature. It should also be determined that levels for synergistic toxicity are not reached. Such factors must be investigated along with temperature to impart confidence to statements of causality. The relative importance of affecting factors to the organisms in a system should be determined by aquatic ecologists who are familiar with the system and who are responsible for the evaluation of that system. Response factors to be specifically mentioned in this paper include those parameters measured for fish, macroinvertebrates, plankton, periphyton, and aquatic macrophytes. Procedures are presented for reducing multiple response factors to unique numerical values which can be used in the appropriate statistical evaluations.

Fish

The count of fish per species is a multiple response measurement. It is desirable to reduce these values to some meaningful composite score, and since certain species are of special value, the score should take this into account. It is assumed that for a given geographic location it is possible to rank the fish species according to their contribution to the economic or esthetic well-being of the area. Those species having the greatest value should be assigned a positive numerical value and those with the least value, a lesser numerical value which may be negative if the species is undesirable. Ties can occur. For example, if a sample yields seven species of fish, three of which are desirable and three not so desirable and one of which is neutral, the simplest scale representing the relative magnitude of importance of these seven species might be 3, 2, 1, 0, -1, -2, -3. The final score used in the analysis would be the sum of the products of the number of fish per species times the scalar value. Under such a plan, locations having a high population of desirable species will get a high score, an evenly mixed location should get a score centering around zero, and a location having a high concentration of undesirable types will get a high negative score. A change in population composition will be readily apparent in the scores.

Other scale values can be chosen. For example, if extensive data are available on the economic value of given fish species, then an economic valued scale can be used. The weight per species and average individual weight in each species are also multiple responses which can be reduced to a composite score using the same scale of importance as given above. Whatever scale is chosen must be used for the duration of the investigation.

Count data are often non-normal; therefore, a nonparametric statistical evaluation, such as the factorial arrangement, non-parametric type given in the Appendix, should be used. A score arrived at by the scale of importance is derived from a count so analysis is by the same nonparametric methods. Two methods of handling count data are given in the Appendix.

The statistical evaluation used is also dependent upon the questions to be answered. If the primary question is whether there has been a gross change over time, then an analysis is made of the composite scores to detect changes in community composition. If the interest is in determining shifts in a given species over time, then an analysis is made for each species. In this case, the average weight/species should be used, and a parametric analysis, as identified in the Appendix, is the proper evaluation tool. In some studies, both methods may be used.

Macroinvertebrates

Standard procedures for presenting benthic data include bar charts or graphs which present the data, but are not always sufficient to show the combined effects of various chemical constituents and hydrographic conditions upon the structure of the aquatic community. In an effort to alleviate this problem, various diversity indices have been developed. Those showing the greatest promise are given by Margalef (35) and are derived from information theory.

Diversity indices are a measure of the species distribution of the individuals of a community. The most diverse community possible would contain a number of species with each having the same number of individuals. The least diverse community would have one species including all individuals.

The index for redundancy (R), often used with the diversity index, is a measure of preponderance of individuals in a few species. Redundancy is greatest with a large number of individuals in one species and least with one individual per species, so R decreases as diversity increases.

The use of diversity and redundancy indices is based upon the theory in ecology that more stable communities are more diverse, i.e., they contain more species and the number of organisms is relatively well distributed among the species. A stable community would have a higher diversity and a lower redundancy value than a less stable community. If the diversity decreases and redundancy increases for the community below a thermal discharge, one would assume that some species are being eliminated and that the system is becoming less stable (36).

Diversity ($\overline{H}$) and redundancy (R) are computed by means of the following equations derived from Wilhm (37).

$$\overline{H} = \frac{1}{N} \left(\log_2 N! - \sum_{i=1}^{S} \log_2 n_i!\right)$$

and

$$R = \frac{\sum_{i=1}^{S} \log_2 n_i! - S \log_2 \left(\frac{N}{S}\right)!}{\log_2 (N-S+1)! - S \log_2 \left(\frac{N}{S}\right)!}$$

where

n_i = Number of organisms in the ith species

$N = \sum_{i=1}^{S} n_i$ = total number of organisms

S = Number of species

Base two logarithm is used.

This gives two values to describe a sample. These may be used as they are or they can be reduced to one response value per sample which is comparable to other samples from the study area. This is done by use of the following modified equation from Kendall (38) for dispersion:

$$D_L = \frac{[K_R - (K_{max} + 1)]^2}{V_R} + \frac{(K_{\overline{H}} - 1)^2}{V_{\overline{H}}}$$

where

L = Location (sampling point) 1, 2,...n

K_R = Rank of R at location L

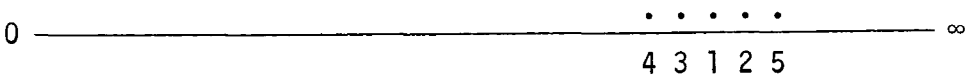
K_{max} = Highest rank of R

$K_{\overline{H}}$ = Rank of $\overline{H}$ at location L

V_R and $V_{\overline{H}}$ = Variance of R and $\overline{H}$, respectively.

A dispersion (D_L) value is computed for each sample at each time of sampling and is a relative measure of the difference between the combination of diversity and redundancy in that sample and in a theoretical sample where $\overline{H} = 0$ and $R = 1$, i.e., where there are no organisms present at all. A sample with a dispersion value of 2.0 would be much closer to the worst case than would a station with a value of 23.0. In comparing data from several locations, if all stations have high values except for those in the plume, then something is decreasing the diversity and is probably detrimental to the resource. In a before-and-after case, we can look for changes in the dispersion values to point out changes in community. The reason(s) for change will be determined from an examination of possible affecting factors.

As an example, in a body of water where physical conditions (bottom type, current, etc.) are fairly uniform, the dispersion values for five stations will fall within a narrow range and "clump" at one point between 0 and infinity.



0 - ∞ = Dispersion (D_L) values (a positive number computed by the equation for D_L)

1 - 5 = Station numbers (L)

If stations are laid out downstream from 1-5 and some form of toxicant (possibly heat) is introduced between stations 1 and 2, the distribution of D_L values would change and could look like this:

This would indicate a drastic change at station 2, but a gradual recovery downstream to station 5, which has nearly the same D_L value as station 1.

To obtain the D_L values, first compute diversity ($\overline{H}$) and redundancy (R) for each station or sample location. The values of $\overline{H}$ are then ranked from lowest to highest. The corresponding R values for each station are ranked from highest to lowest since R decreases as $\overline{H}$ increases. Since D_L is a measure of difference between each station and a control value, a control point is necessary to ensure a common starting point for subsequent comparisons. The control value for the $\overline{H}$ values is arbitrarily given a rank of 1, so $\overline{H}$ values for sample stations rank from 2 on. The control value for R is one greater than the number of stations being compared.

The variances (V_R and $V_{\overline{H}}$) for each station, but not the control, are computed from the ranked values by using the equation:

$$\text{Var } (R \text{ or } \overline{H}) = \frac{1}{12n} [(n^3 - n) - \sum(t^3 - t)]$$

where

n = Number of observations

t = Number of tied ranks.

For example, if the following values are obtained,

		Locations or station numbers					
		L_1	L_2	L_3	L_4	L_5	Rank of Control Value
Computed Values	R	.61	.49	.37	.22	.20	
	$\overline{H}$	.98	1.34	2.45	3.25	3.25	
Ranks	R	5	4	3	2	1	6
	$\overline{H}$	2	3	4	5.5	5.5	1

then for L_1:

$$V_R = \frac{1}{12(5)} [(5^3 - 5) - 0] = 2.00$$

$$V_{\overline{H}} = \frac{1}{12(5)} [(5^3 - 5) - (2^3 - 2)] = 1.81$$

Comparing the control value to location 1 by means of the equation for D_L gives:

$$D_1 = \frac{(5 - 6)^2}{2} + \frac{(2 - 1)^2}{1.81} = \frac{1}{2} + \frac{1}{1.81} = .5 + .55 = 1.05,$$

and comparing the control value to location 5 gives:

$$D_5 = \frac{(1 - 6)^2}{2} + \frac{(5.5 - 1)^2}{1.81} = \frac{25}{2} + \frac{20.25}{1.81} = 23.75.$$

The other values are computed the same way. These dispersion values can then be plotted on a line from 0-24 for a graphic display of the differences between stations.

It may be superfluous to use any statistical technique to check for population shifts once the dispersion values are computed. However, a nonparametric method of either type discussed in the Appendix is recommended if a test is desired.

In the marine environment, or in any situation where it is possible to establish a relative value for various benthic species, a scale of importance, such as the one recommended for fish data, may be used for an indication of whether species shifts are beneficial or detrimental to the system or to the economics of a particular region.

Plankton, Periphyton and Aquatic Macrophytes

Data for these three groups will be presented, in part, at least, as percentages. Plankton and periphyton may be weighed for a measure of biomass, but there will also be an estimate of percent total composition of algal types (green, blue-green, etc.). Aquatic macrophyte data will generally be in terms of percent plant coverage of an area.

For replicated data, the percentage values should be normalized using the arc sin transformation so that standard statistical tests can be used. This is accomplished by referring the percentage value to the arc sin table in Steele and Torrie (39) or any other statistics text. If done by computer, the equation: angle in degrees = arc sin $\sqrt{\text{percent}}$ can be used for the transformation. Arc sin values may be expreseed as degrees or radians since one is a multiple of the other.

For replicated data, the experimental error used in the tests for significance should be calculated from the data. For unreplicated data, the following method can be used to determine an experimental error for the tests. This method gives the minimum experimental error which is usually lower than that calculated from replicated data.

If the transformed value is reported in degrees, the theoretical experimental error is 821/n and if in radians the error is 0.25/n. The divisor, n, is the common denominator of all the fractions used to compute the percentages. For example: if 200 microscope grid squares are viewed on a periphyton-covered glass plate and 100 squares are covered with green algae, the percentage is calculated as 100/200 = 50 percent. The denominator is 200 and the theoretical error term in degrees is 821/200. In radians, it is .25/200. Although use of the error term is explained in the Appendix in the section on Analysis of Variance (Parametric), it is included here to point out the importance of using equal areas or volumes for each sample from which a percentage is obtained. Unequal volumes or areas change the denominator so the theoretical error term used in the statistical analyses is not constant.

STATISTICAL ANALYSIS

Experimental design includes consideration of sampling plan, sampling methods and the statistical techniques to be used for analysis of the resulting data. This section is devoted to analysis techniques; the first two components have been discussed in previous sections.

Investigators should design the ideal experiment, then examine it in light of the deficiencies inherent in the actual field situation. If the field survey plan violates the basic assumptions of controlled experimental design, then revisions are required. The following techniques do not violate the assumptions of randomization of experimental units (i.e., fish, benthic organisms, etc.) in the system nor the assumption of replication necessary to obtain a valid estimate of experimental error. Random sampling is not to be confused with haphazard sampling. The idea is to obtain a random sample, representative of the experimental unit(s) of interest at a preselected point. This is commonly referred to as stratified random sampling (40).

While the sampling program is an essential aspect of the overall study, the final result is dependent upon the data analysis. There are a number of statistical techniques which may be used in handling biological data and proper use of one or more of the suitable methods is needed before interpretation of field study data can be accepted.

This paper presents only two of a large number of basic statistical techniques which could be used. However, the two methods selected should enable the user to deal with the wide variety of data associated with the biological responses to thermal discharges. For other methods, consult a statistician and any of the statistical references given.

The basic technique to be used is the factorially arranged analysis of variance (or covariance). Seasonal effects exist, but it is assumed that there is no real seasonal interaction with treatment so any apparent interaction between seasons and treatments is a measure of the experimental error. Factors which can be considered as entering at random, but physically controlled, are transect location, depth of sample collection, and stream width location. These factors define the statistical strata to be sampled. Those considered to be entering at random, but not physically controlled, include velocity, flow rate, temperature, D. O., and the other chemical parameters. These constitute the set of factors upon which the response factor is regressed. The response factor (number of fish, diversity, etc.) is measured to detect yearly changes between pre- and post-construction periods or between sampling locations.

If only the physically controlled factors are considered in the statistical analysis, then a factorial experiment, randomized complete block analysis of variance, is used. General methods for analyzing parametric as well as nonparametric data are given and identified in the Appendix. For more thorough discussions of the parametric analysis, consult the texts by Steele and Torrie (39), Cochran and Cox (40), or Winer (41). The nonparametric factorial method is discussed more thoroughly in Wilson (42).

If the physically uncontrollable but measurable effects are to be used in the evaluation, then a factorial experiment: randomized complete block analysis of covariance can be used. The parametric analysis is discussed in Winer (41) and in Steele and Torrie (39). The nonparametric analysis of covariance is discussed in the paper by Quade (43). Summaries of these analyses are given in the Appendix along with discussions of their use, extensions, and interpretations.

A factorially arranged experiment allows the examination of synergistic effects between the factors included in the model as well as examination of each factor by itself. Failure to include the interaction inflates the error term and thus masks significant effects that exist.

Isolating and examining the effects of covariates (physically uncontrollable but measurable factors) is aided by performing a series of step-wise analyses of covariance and examining the net effect upon the adjusted and unadjusted F values obtained. There are computer programs available to do these calculations.

The interpretation of results obtained from either of the above-mentioned analyses is dependent upon the number of possible explanations presented by data collected from the system being studied and upon the knowledge and experience of the persons conducting the study. Alone, statistical analysis of a limited set of biologic data can lead to spurious "conclusions"; on the other hand, a purported biological effect cannot be confidently accepted unless it can stand the test of statistical significance. In all cases, statistics and reason must be combined to arrive at the best possible conclusions.

APPENDIX

The appendix describes the factorial arrangement for analysis of variance (AOV) and analysis of covariance (AOCV) techniques for both parametric and nonparametric data. In some cases, numerical examples are given to clarify the procedures. The aim is to show the reader how to set up the analyses, arrange the data, make the necessary computations, and understand the meaning of the results. In an actual situation, the amount of data would likely be so large that a computer would be necessary. Still, the basic techniques illustrated by the following examples would apply.

The Factorial Arrangement: Analysis of Variance (Parametric)

A factorial experiment allows the simultaneous examination of the effect of a number of different factors. The analysis of variance is based upon the fact that any system has a total variability which can be partitioned or divided into portions attributable to different factors recognized in the model. An additional, unexplainable part of the variability is called random variation, or the error term. The method for dividing the total sum of squares used to obtain these variances in a system can best be explained by use of examples so a brief general explanation is given and then a simple numerical example is presented so the statistical test can be carried to a conclusion.

Consider the river situation. In this case, the following factors and levels of factors are included:

Time at 2 levels: Pre- and post-construction.

Transects at 4 levels: Above outfall, immediately below outfall, 0.5 ΔT below outfall, and 0.1 ΔT below outfall.

Width at 3 levels for fish (at bank, midstream, and left bank) and at 4 levels for benthos, chemical data, etc.

Depth at 3 levels for chemical analyses (not considered in biological analyses).

Seasons form four blocks.

A general diagram of the data arrangement for one block is given in Table A-1. A portion of this table has been filled in for the spring block of data collected. Any response, R, can be uniquely identified by the proper use of subscripts to denote season, transect, width, time, and replicate.

Blocks (seasons) = B_h where h = 1, 2, 3, 4 (Spring, Summer, Fall, and Winter).

Transect = T_{hi} where i = 1, 2, 3, 4, (above, below, 0.5 ΔT, and 0.1 ΔT).

Width = W_{hj} where j = 1, 2, 3, for fish (left, middle, right) or 1, 2, 3, 4, for benthos (0.1W, 0.3W, 0.7W, 0.9W).

Construction Time = C_{hk} where k = 1, 2, (before, after).

Response = R_{hijkl} where l = 1, 2 (first, second replicate)

For example: $R_{2\ 3\ 2\ 2\ 1}$ is taken in the summer at transect 0.5 ΔT in the center section of the stream (for fish) after construction of the plant and is the first replicate.

TABLE A-1

RAW DATA SHEET

Spring (Block 1)								
Width (W) / Transect (T)	Construction Time (C)	1	2	3	4	T_{sum}		Grand Sum
1	1	$R_{1,1,1,1,1}$	$R_{1,1,2,1,1}$			T_{11}	REPEAT FOR SUMMER, FALL, WINTER (BLOCKS - 2,3,4)	$GT_1 = \sum_{h=1}^{4} T_{h1}$
		$R_{1,1,1,1,2}$	$R_{1,1,2,1,2}$					
		Rep Sum	Rep Sum					
	2	$R_{1,1,1,2,1}$						
		$R_{1,1,1,2,2}$						
		Rep Sum						
2	1					T_{12}		GT_2
	2							
3	1					T_{13}		GT_3
	2							
4	1					T_{14}		GT_4
	2							
CW Sum		$C_{11}W_{11}$	$C_{11}W_{12}$	$C_{11}W_{13}$	$C_{11}W_{14}$	C_{11}		$GC_1 = \sum_{h=1}^{4} C_{h1}$; G_{tot} =
		$C_{12}W_{11}$	$C_{12}W_{12}$	$C_{12}W_{13}$	$C_{12}W_{14}$	C_{12}		GC_2 ; $\sum GT = \sum GW =$
W Sum		W_{11}	W_{12}	W_{13}	W_{14}	Blk. tot. (BT)		GW_1, GW_2, GW_3, GW_4 ; $\sum GC$

TABLE A-2

SAMPLE PROBLEM RAW DATA SHEET (WEIGHTS PER UNIT AREA SAMPLED)

	Block 1 - Spring						Block 2 - Summer						
Width / Transect	Const. Time	1 (0.1 W)	2 (0.3 W)	3 (0.7 W)	4 (0.9 W)	T_{sum}	Const. Time	1	2	3	4	T_{sum}	Grand Sums
1 (above)	1 (before)	6,10 16	7,10 17	5,11 16	8,9 17		1	12,13 25	11,13 24	10,13 23	12,12 24		
	2 (after)	9,8 17	7,10 17	8,7 15	9,7 16	131	2	14,12 26	11,11 22	14,9 23	13,12 25	192	323
2 (below)	1	10,8 18	7,9 16	6,11 17	8,8 16		1	12,12 24	11,14 25	10,13 23	11,14 25		
	2	0,1 1	2,1 3	8,7 15	7,8 15	101	2	0,1 1	3,2 5	12,12 24	13,10 23	150	251
3 (0.5 ΔT)	1	7,9 16	9,9 18	9,8 17	8,7 15		1	10,9 19	10,11 21	11,14 25	11,11 22		
	2	4,3 7	5,4 9	8,8 16	9,9 18	116	2	4,5 9	7,6 13	10,13 23	12,12 24	156	272
4 (0.1 ΔT)	1	9,9 18	7,9 16	8,7 15	8,8 16		1	11,13 24	13,10 23	9,12 21	13,13 26		
	2	11,10 21	10,9 19	9,9 18	8,9 17	140	2	9,8 17	10,9 19	12,12 24	12,13 25	179	319
CW Sum		68 46	67 48	65 64	64 66	264 224		92 53	93 59	92 94	97 97	374 303	638 527
W Sum		114	115	129	130	488		145	152	186	194	677	259 267 315 324 1165

Table A-2 is the same as A-1 except that the squares are filled with whole numbers which have been chosen to represent average weights of benthic invertebrates of a species. In a real situation, the weights could be in grams or any other measure of weight. The numbers in the example were chosen to avoid decimals and make the example simple. Note also that since these are benthic data, there are four widths and not three as would be the case if the table were set up for fish data. The example is carried out for only two seasons, again for simplification. The method of obtaining the sums should be evident from the table. If it is not, any of the referenced statistics books can be consulted.

The numbers in the example were chosen specifically to illustrate possible biological changes after operation of a power plant in a hypothetical situation illustrated by Figure 3.

In order to compute the sums of squares for the factors under consideration, Interaction Table A-3 should be prepared. Note that only single subscripts are used in Table A-3, since the block (i.e., season) subscript, h, has been removed by summing across blocks. The entries in this table are obtained by first summing across replicates and blocks in Table A-1 or A-2 to obtain the T_i, C_k, and W_j entries where $T_i = \sum R$ for transect i; $C_k = \sum R$ for time; $W_j = \sum R$ for widths. For example, $T_1C_1W_1$ is the sum of the spring, summer, fall and winter values for both replicates

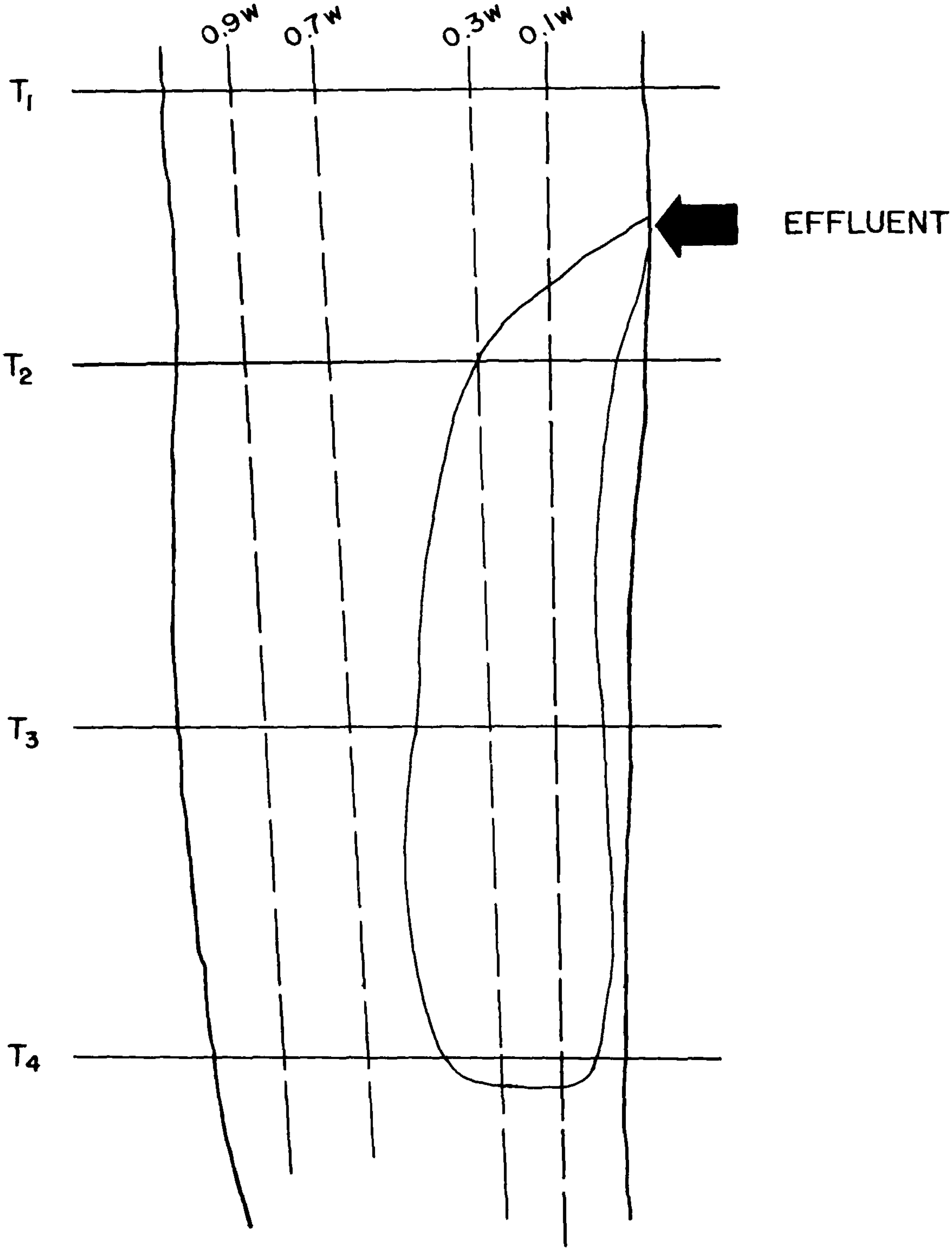


FIGURE 3. SAMPLING GRID AND HEATED AREA FOR EXAMPLE PROBLEM.

and all blocks for transect 1, time 1 (before construction), and width 1. Adding across in Table A-3 gives the sums T_iC_k and down rows within transects gives intermediate sums T_iW_j. Adding down rows within time frames gives the sums C_kW_j. Adding across columns of intermediate row sums (T_iW_j values) gives the GT_i values. Adding $C_1W_j + C_2W_j$ gives the GW_j values and adding $C_kW_1 + C_kW_2 + C_kW_3 + C_kW_4$ gives the GC_k values. The sums of the GT_i, GW_j, and GC_k values should all be equal to their respective grand total values for the corresponding factors in Table A-1. If they are not, an error in addition has occurred.

Table A-4 illustrates numerically the development of an interaction table (i.e., Table A-3). Data from Table A-2 were used to construct Table A-4.

Sums of squares are computed in the following manner:

$$\text{Total sum of squares} = \sum_{hijkl} R^2_{hijkl} - GS = TTSS, \text{ where } GS = (GTot)^2/N$$

$$\text{Block sum of squares} = \sum_h \frac{(BT)_h^2}{N_B} - GS = BSS$$

$$\text{Transect sum of squares} = \sum_i \frac{(GT)_i^2}{N_T} - GS - TSS$$

$$\text{Width sum of squares} = \sum_j \frac{(GW_j)^2}{N_W} - GS = WSS$$

$$\text{Construction sum of squares} = \sum_k \frac{(GC_k)^2}{N_C} - GS = CSS$$

TABLE A-3

INTERACTION TABLE

Transect	Time	Width				Total
		W_1	W_2	W_3	W_4	
T_1	C_1	$T_1C_1W_1$	$T_1C_1W_2$	$T_1C_1W_3$	$T_1C_1W_4$	T_1C_1
	C_2	$T_1C_2W_1$	$T_1C_2W_2$	$T_1C_2W_3$	$T_1C_2W_4$	T_1C_2
TOTAL		T_1W_1	T_1W_2	T_1W_3	T_1W_4	GT_1
T_2	C_1	$T_2C_1W_1$	$T_2C_1W_2$	$T_2C_1W_3$	$T_2C_1W_4$	T_2C_1
	C_2	$T_2C_2W_1$	$T_2C_2W_2$	$T_2C_2W_3$	$T_2C_2W_4$	T_2C_2
TOTAL		T_2W_1	T_2W_2	T_2W_3	T_2W_4	GT_2
T_3	C_1	$T_3C_1W_1$	$T_3C_1W_2$	$T_3C_1W_3$	$T_3C_1W_4$	T_3C_1
	C_2	$T_3C_2W_1$	$T_3C_2W_2$	$T_3C_2W_3$	$T_3C_2W_4$	T_3C_2
TOTAL		T_3W_1	T_3W_2	T_3W_3	T_3W_4	GT_3
T_4	C_1	$T_4C_1W_1$	$T_4C_1W_2$	$T_4C_1W_3$	$T_4C_1W_4$	T_4C_1
	C_2	$T_4C_2W_1$	$T_4C_2W_2$	$T_4C_2W_3$	$T_4C_2W_4$	T_4C_2
TOTAL		T_4W_1	T_4W_2	T_4W_3	T_4W_4	GT_4
TOTAL		C_1W_1	C_1W_2	C_1W_3	C_1W_4	GC_1
TOTAL		C_2W_1	C_2W_2	C_2W_3	C_2W_4	GC_2
TOTAL		GW_1	GW_2	GW_3	GW_4	
						G Tot.

TABLE A-4

SAMPLE PROBLEM INTERACTION TABLE (WEIGHTS PER UNIT AREA SAMPLED)

Transect	Time	0.1 W	0.3 W	0.7 W	0.9 W	Total
T_1	C_1	16+25=41	41	39	41	162
	C_2	43	39	38	41	161
TOTAL		84	80	77	82	323
T_2	C_1	18+24=42	41	40	41	164
	C_2	2	8	39	38	87
TOTAL		44	49	79	79	251
T_3	C_1	35	39	42	37	153
	C_2	16	22	39	42	119
TOTAL		51	61	81	79	272
T_4	C_1	42	39	36	42	159
	C_2	38	38	42	42	160
TOTAL		80	77	78	84	319
TOTAL	C_1W	160	160	157	161	638
TOTAL	C_2W	99	107	158	163	527
TOTAL	GW	259	267	315	324	
						1165

The interaction sums of squares are computed from Table A-3 or A-4. (Note: The main effects sums of squares could also have been calculated from the marginal totals in Table A-2.)

$$\text{TW sum of squares} = \frac{\sum_{ij} (T_i W_j)^2}{N_{TW}} - \text{TSS} - \text{WSS} - \text{GS} = \text{TWSS}$$

$$\text{TC sum of squares} = \frac{\sum_{ik} (T_i C_k)^2}{N_{TC}} - \text{TSS} - \text{CSS} - \text{GS} = \text{TCSS}$$

$$\text{WC sum of squares} = \frac{\sum_{jk} (C_k W_j)^2}{N_{CW}} - \text{CSS} - \text{WSS} - \text{GS} = \text{WCSS}$$

$$\text{TWC sum of squares} = \frac{\sum_{ijk} (T_i W_j C_k)^2}{N_{TWC}} - \text{TSS} - \text{CSS} - \text{WSS} - \text{TWSS}$$

$$- \text{TCSS} - \text{WCSS} - \text{GS} = \text{TWCSS}$$

$$\text{Experimental error sum of squares} = \frac{\sum_{hijk} (\text{Rep Sum})^2}{N_R} -$$

$$\text{GS} - \text{BSS} - \text{TSS} - \text{WSS} - \text{CSS} - \text{TWSS} - \text{TCSS} - \text{WCSS} - \text{TWCSS} = \text{EESS}$$

$$\text{Sampling error of squares} = \sum_{hijkl} R^2_{hijkl} - \frac{\sum_{hijk} (\text{Rep Sum})^2}{N_R} = \text{SESS}$$

Experimental error is the sampling error plus the interaction between the replicate factor and blocking factor, whereas sampling error is a measure of the lack of agreement among observations from the same treatment cell.

The divisors in the above error equations (N_R) can be obtained by counting the number of observations which were added to get each of the values in the numerator. This can become laborious; therefore, methods for calculating them have been developed. For the numerical example given, let the number of blocks used be called "b" (2), the number of replications be "r" (2), the number of transects be "t" (4), the number of widths be "w" (4), and the number of time periods be "c" (2). Then the total number of observations, N, is equal to brtwc or $2 \times 2 \times 4 \times 4 \times 2 = 128$; N_B (the number of observations per block) is rtwc or $2 \times 4 \times 4 \times 2 = 64$; N_T (number of observations per transect) is brwc or $2 \times 2 \times 4 \times 2 = 32$; N_W (number of observations per width) is brtc or $2 \times 2 \times 4 \times 2 = 32$; N_C (number of observations per time period) is brwt = 64; N_{TW} (number of observations at each transect and width) is brc = 8; N_{TC} = brw = 16; N_{CW} = brt = 16; and N_{TWC} = br = 4; N_R = 2.

Using the hypothetical data presented in Tables A-2 and A-4. the sums of squares are computed by the equations just given:

$$TTSS = 11{,}859 - \frac{1165^2}{128} = 11{,}859 - 10{,}603 = 1{,}256$$

$$BSS = \frac{488^2 + 677^2}{64} - 10{,}603 = 279$$

$$TSS = \frac{323^2 + 251^2 + 272^2 + 319^2}{32} - 10{,}603 = 118$$

$$WSS = \frac{259^2 + 267^2 + 315^2 + 324^2}{32} - 10{,}603 = 102$$

$$CSS = \frac{638^2 + 527^2}{64} - 10{,}603 = 97$$

$$TWSS = \frac{87{,}521}{8} - 118 - 102 - 10{,}603 = 117$$

$$TCSS = \frac{175{,}081}{16} - 118 - 97 - 10{,}603 = 125$$

$$WCSS = \frac{174{,}553}{16} - 97 - 102 - 10{,}603 = 108$$

$$TWCSS = \frac{45{,}483}{4} - 118 - 97 - 102 - 117 - 125 - 108 - 10{,}603 = 101$$

$$EESS = \frac{23,459}{2} - 10,603 - 279 - 118 - 102 - 97 - 117 - 125 - 108 - 101 = 80$$

$$SESS = 11,859 - 11,730 = 129$$

As a check on the computations, the TTSS computed initially can be compared to:

$$TTSS = BSS + TSS + WSS + CSS + TWSS + TCSS + WCSS + TWCSS + EESS + SESS = 1,256$$

The results from the above calculations are presented in an analysis of variance table, Table A-6, which follows the form of a general AOV table (A-5). The F values obtained are compared to tabular F values from F tables with the given numerator and denominator degrees of freedom at a selected level of significance. It should be noted that if this analysis or a similar one containing block effects is used, it is not strictly necessary to have replication. If replication is not done, then EEMS is used in all tests and no measure of true experimental error is obtained. However, if comparisons between species at given stations or transects are contemplated, replication is required to give a valid estimate of the error rate in the system.

TABLE A-5

ANALYSIS OF VARIANCE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F
Total	rbtwc - 1	TTSS		
Blocks	b - 1	BSS		
Transect	t - 1	TSS	TMS=TSS÷df	TMS÷EMS*
Width	w - 1	WSS	WMS=WSS÷df	WMS÷EMS*
Construction Time	c - 1	CSS	CMS=CSS÷df	CMS÷EMS*
TW	(t-1)(w-1)	TWSS	TWMS=TWSS÷df	TWMS÷EMS*
TC	(t-1)(c-1)	TCSS	TCMS=TCSS÷df	TCMS÷EMS*
WC	(w-1)(c-1)	WCSS	WCMS=WCSS÷df	WCMS÷EMS*
TWC	(t-1)(w-1)(c-1)	TWCSS	TWCMS=TWCSS÷df	TWCMS÷EMS*
Experimental Error	(b-1)(twc-1)	EESS	EEMS=EESS÷df	EEMS/SEMS
Sampling Error	btwc(r-1)	SESS	SEMS=SESS÷df	

*If EEMS/SEMS is significant, then EMS is not significantly different from EEMS; if it is not significant, then EMS is not significantly different from SEMS. For more detailed discussion, see Steele and Torrie (39).

TABLE A-6

SAMPLE PROBLEM ANALYSIS OF VARIANCE

Source	DF	SS	MS	Computed F	Tabular F*
Total	128 - 1 = 127	1256	--	--	--
Blocks	2 - 1 = 1	279	--	--	--
Transect	4 - 1 = 3	118	39	19.5	2.7
Width	4 - 1 = 3	102	34	17.0	2.7
Const. Time	2 - 1 = 1	97	97	48.5	4.0
TW	3 × 3 = 9	117	13	6.5	2.0
TC	3 × 1 = 3	125	42	21.0	2.7
WC	3 × 1 = 3	108	36	18.0	2.7
TWC	3 × 3 × 1 = 9	101	11	5.5	2.0
Exp. Error	(2-1)(4×4×2-1)=31	80	2.6	1.3	1.6**
Sample Error	(2×4×4×2)(2-1)=64	129	2.0		

*Tabular F ratios were selected at the 5% level of significance.

**Since the $F = \frac{EEMS}{SEMS}$ is not significant (i.e., $1.3 < 1.6$), then EMS is not statistically significantly different from SEMS. That is, there is no block by response interaction.

For the analysis of variance, the F test is one-tailed on the right. Therefore, when the computed F ratio exceeds the tabular value, the variation due to the effect being tested is statistically significant. If the computed F ratio is less than the tabular F, the variation is not statistically significant.

Thus, it is seen from Table A-6 that the variations in biomass of benthic invertebrates due to all main effects and all interaction effects are statistically significant. In other words, the variations are greater than would be expected from experimental or sampling error.

In terms of the hypothetical heated water discharge, this means that there was a statistically significant change in biota after introduction of the heated discharge. The following significant effects can be seen from Table A-6:

1. There was a difference in biota among transects. This is reasonable since three transects are within the heated zone and one is outside (See Figure 3).

2. There was a difference in biota at different stations based on percent of river width. Only one side of the river is covered by the heated water.

3. There was a difference attributable to construction time. Invertebrate biomass decreased significantly after plant operation began.

4. There was an interaction between transect and location by width. Each sample station is located at the intersection of a transect line and a line of percent width. Samples at intersections outside the heated area were different from those inside.

5. There was an interaction between transect and construction time and between location by width and construction time. There was also an interaction between all three. Transect and location by width determine location of a sample and construction time determines whether the sample point is covered by heated waters.

The statistical analysis is not the whole story, but this information can be combined with graphic presentations of the data to form a composite picture. The statistical tests are important to show whether apparent changes are significant.

In the test on Table A-6, the F value was arbitrarily chosen at the 5 percent level of significance to illustrate the point of the sample problem. This is a commonly accepted level, but in an actual field study where results are not significant at the 5 percent level the F may be varied to determine that level at which significance is found. Then, the F is chosen such that the tabular F value is that value in the table which is under, but closest to the computed F ratio. This will show at which level the variation due to a specific factor is significant. Of course, the results may be accepted with much more confidence at the 1-5 percent level than at the 30-40 percent level.

Sample Size Estimation and Confidence Intervals

The first year's data can be used to determine if replication is adequate for making decisions. Following are two methods for arriving at required sample size (39, 40).

These calculations depend upon:

1. An estimate of σ^2, the true experimental error,
2. The size of the difference to be detected, δ or d,
3. The assurance with which it is desired to detect the difference (Type II error or β),
4. The level of significance to be used in the actual experiment (Type I error or α), and
5. Whether a one-tailed or two-tailed test is required.

The following equation is an approximation since it assumes

$$S^2 = \sigma^2 \quad r \geq \frac{2(t_0 + t_1)^2 \; S^2}{\delta^2}$$

where

r = Number of replicates

S^2 = Best available estimate of σ^2

δ = Difference of practical importance to be detected

t_0 = Student's t associated with Type I error (α, rejection of a true hypothesis)

t_1 = Student's t associated with Type II error (β, acceptance of a false hypothesis) or the probability of detecting δ if it exists.

Use the table for t_0 for error degrees of freedom at the α level and t_1 for error degrees of freedom at $2(1 - \beta)$ level.

Assume the statistical model to be used fits Table A-6. There will be four seasons (blocks), four transects, four width measuring points, six years (three pre- and three post-), and two replicates

Assume that a 30 percent average weight reduction is considered a change of practical importance. The Type I error is $0.05 = \alpha$ and the Type II error is $0.10 = \beta$.

The error degrees of freedom in the completed experiment is given by $btwc(r - 1) = 4 \times 4 \times 4 \times 6(2 - 1) = 384$. The mean weight from Table A-4 is 9.1, therefore δ is $0.3 \times 9.1 = 2.7$ grams. Then,

$$r \geq \frac{2(1.96 + 0.842)^2 \ (2.0/4)}{2.7^2} \approx 1.1 \text{ or } 2.$$

The value 2.0/4 is used since a test for reduction about the mean is desired and four is the smallest number of means to be compared in the ANOVA. Since a one-tailed test is indicated by the term "reduction," the t values are taken at $\alpha/2$ and $\beta/2$. The value computed is always rounded up.

To obtain sample size sufficient to compute a confidence interval no larger than a specified size, use the equation

$$r = \frac{S^2 \ q_\alpha^2 \ (P,n_2) \ F_\gamma \ (n_2,n_1)}{d^2}$$

where

S^2 = Variance of the smallest number of means to be compared (4)

q_α = Value from the studentized range table for four means (P) and degrees of freedom equal to the error degrees of freedom from the final survey (n_2)

F_γ = Value of Snedecor's F at the γ significance level for n_2 degrees of freedom and n_1 (current sample size) degrees of freedom.

d = Half-length confidence interval - again taken as 30 percent of the overall mean, or 2.7 grams.

This gives

$$r = \frac{0.5\,(3.02)\;(1.0)}{2.7^2} \simeq 1.0$$

This indicates that replication is not required; however, one should refrain from obtaining fewer than two replicate samples.

Methods for setting confidence intervals are to be found in any of the parametric statistical texts referenced.

The Factorial Arrangement: Analysis of Variance (Nonparametric)

If the raw data is in the form of counts, or if it is badly skewed, it is often desirable to analyze it by nonparametric techniques. The procedure by Wilson (42) has proved to be very satisfactory for evaluating counting data in water quality studies In this procedure, the chi-square statistic for a contingency table is broken into components in much the same way as a total sum-of-squares is decomposed in analysis of variance computations. This provides a nonparametric test of the hypothesis concerning main effects and interaction much like the parametric test using two-way analysis of variance.

Wilson explains use of the test for two factors with interaction to include more than two factors and expands this test in his paper (42). Siegel (44) gives methods for cases where interactions are not of interest. A simple example of the factorial test is given in the section to follow; however, the more basic references will have to be consulted for application to a large-scale sampling program.

In the sampling program proposed in this paper, there are five factors: blocks (seasons), replications, transects, widths, and time (pre- and post-construction). In the actual survey comprised of six years data, time will be in years and a special test will have to be made for a strict pre- and post-construction test. In the sampling problem to be presented, only transect and time are considered for simplicity. In an actual situation, the method becomes so awkard that a computer is necessary to handle the table construction and analyses.

For the sample problem, we assume that two sets (series) of fish samples are taken before plant operation and two after operation on each of the four transects shown in Figure 3. Since there are three stations per transect, the total number of samples is: 2 times (before and after construction) × 2 series per time × 4 transects × 3 stations per transect = 48. The data represent total fish counts per transect.

The following step-wise process is required to complete the analysis. First, compute the median value for the entire set of data. This divides the data into two sets with nearly equal numbers of observations where n_a is the number of samples equal to or greater than the median and n_b is the number of samples less than the median. For example, if the numbers of fish caught per station vary from one to 100, the median value could be 46. If so, half of the samples have 46 or more fish and the other half have fewer than 46 fish.

This information is set up in a two-way contingency table such as Table A-7 which considers only transect and construction time. The cell entries in the table are the numbers of observations taken before and after construction on each transect. They are divided into two groups depending upon whether the number of fish caught is equal to or greater than the median or less than the median. For example, in transect one, 14 samples before construction had a number of fish equal to or greater than the median number of fish per sample. In this case, 14 samples had 46 or more fish.

TABLE A-7

CONTINGENCY TABLE FOR NONPARAMETRIC AOV

Sample Size	Time \ Transect	1	2	3	4	Total $_af_{i\cdot}$
≥ Median	Before	14	16	16	14	60
	After	9	6	8	13	36
$_af_{\cdot j}$		23	22	24	27	96
						$_bf_{i\cdot}$
< Median	Before	10	8	8	10	36
	After	15	18	16	11	60
$_bf_{\cdot j}$		25	26	24	21	96
$_af_{\cdot j} + {_bf_{\cdot j}}$		48	48	48	48	192

Ten of the before samples in transect one had fewer than the median number of fish.

Certain checks can be made to ensure that counting errors are not made. The total number of samples in each transect should equal 48 with 24 samples taken before construction and 24 taken after.

The information in the contingency Table A-7 will be used to perform three chi-square tests to determine whether:

1. There is significant overall variability in the data,
2. There is a significant difference between before and after data,
3. There is a significant difference between transects.

The total chi-square value for test 1 is computed from the following equation:

$$\chi^2_T = \sum_i \sum_j \left[\frac{({}_af_{ij} - n_{ij}n_a/n)^2}{n_{ij}n_a/n} + \frac{({}_bf_{ij} - n_{ij}n_b/n)^2}{n_{ij}n_b/n} \right]$$

where

${}_af_{ij}$ = Represents the number of observations in the cell in row i and column j which are equal to or greater than the median

$_b f_{ij}$ = Represents the number of observations in the cell in row i and column j which are less than the median

$$n_a = \sum_i \sum_j {}_a f_{ij}$$

$$n_b = \sum_i \sum_j {}_b f_{ij}$$

$$n_{ij} = {}_a f_{ij} + {}_b f_{ij}$$

$$n_a = n_b = n/2$$

For the numerical computation:

$$_a f_{ij} = 14,\ 9,\ 16\ \ldots\ 13$$

$$_b f_{ij} = 10,\ 15,\ 8\ \ldots\ 11$$

$$n_{ij} = 24$$

$$n_a = 96 \qquad n_b = 96$$

$$n = 192$$

$$n_{ij}\ n_a/n = 24 \times \frac{96}{192} = 12$$

$$n_{ij}\ n_b/n = 24 \times \frac{96}{192} = 12$$

$$\chi^2_T = \frac{(14-12)^2+(16-12)^2+(16-12)^2+(14-12)^2+(9-12)^2+(6-12)^2+(8-12)^2+(13-12)^2}{12}$$

$$+ \frac{(10-12)^2+(8-12)^2+(8-12)^2+(10-12)^2+(15-12)^2+(18-12)^2+(16-12)^2+(11-12)^2}{12}$$

$$= \frac{2^2 + 4^2 + 4^2 + 2^2 + 3^2 + 6^2 + 4^2 + 1^2}{12}$$

$$+ \frac{2^2 + 4^2 + 4^2 + 2^2 + 3^2 + 6^2 + 4^2 + 1^2}{12}$$

$$= \frac{4+16+16+4+9+36+16+1}{12} + \frac{4+16+16+4+9+36+16+1}{12}$$

$$= \frac{102}{12} + \frac{102}{12} = \frac{204}{12} = 17.0$$

χ^2_T has (rc - 1) degrees of freedom

where

$$r = 2$$

$$c = 4.$$

So, (rc - 1) = 8-1 = 7.

At 0.05 level of significance $\chi^2_7 = 14.1$.

Since the calculated χ^2_T of 17.0 is greater than the table χ^2_T of 14.1, we conclude that the total variability among the samples is significant.

The row chi-square value for test (2) is computed from the following equation:

$$\chi^2_R = \sum_i \left[\frac{({}_a f_{i\cdot} - n_{i\cdot} n_a/n)^2}{n_{i\cdot} n_a/n} + \frac{({}_b f_{i\cdot} - n_{i\cdot} n_b/n)^2}{n_{i\cdot} n_b/n} \right]$$

where

$$n_{i\cdot} = \sum_{j \atop i} n_{ij}.$$

For the numerical computation:

$${}_a f_{i\cdot} = 36,\ 60$$

$${}_b f_{i\cdot} = 60,\ 36$$

$$n_{i\cdot} = 96$$

$$\frac{n_a}{n} = \frac{n_b}{n} = \frac{96}{192} = \frac{1}{2}$$

$$\frac{n_{i\cdot}n_a}{n} = \frac{n_{i\cdot}n_b}{n} = 96 \times \frac{96}{192} = 48$$

$$\chi^2_R = \frac{(36-48)^2}{48} + \frac{(60-48)^2}{48} + \frac{(60-48)^2}{48} + \frac{(36-48)^2}{48}$$

$$= \frac{144}{48} + \frac{144}{48} + \frac{144}{48} + \frac{144}{48} = 3 + 3 + 3 + 3 = 12$$

χ^2_R has (r - 1) degrees of freedom

where

$r = 2$

$r-1 = 1.$

At a 0.5 level of significance,

$$\chi^2_1 = 3.8.$$

Since the calculated χ^2_R of 12.0 is greater than the table χ^2_R of 3.8, we conclude that the difference between samples taken before construction and after construction is significant.

The column chi-square value for test (3) is computed from the following equation:

$$\chi^2_C = \sum_j \left[\frac{({}_a f_{\cdot j} - n_{\cdot j} n_a / n)^2}{n_{\cdot j} n_a / n} + \frac{({}_b f_{\cdot j} - n_{\cdot j} n_b / n)^2}{n_{\cdot j} n_b / n} \right]$$

where

$$n_{\cdot j} = \sum_i n_{ij}.$$

For the numerical computation:

$${}_a f_{\cdot j} = 25, 26, 24, 21$$

$${}_b f_{\cdot j} = 23, 22, 24, 27$$

$$n_{\cdot j} = 48$$

$$\frac{n_a}{n} = \frac{n_b}{n} = \frac{48}{96} = \frac{1}{2}$$

$$\frac{n_{\cdot j} n_a}{n} = \frac{n_{\cdot j} n_b}{n} = 48 \times \frac{48}{96} = 24$$

$$\chi^2_C = \frac{(25-24)^2 + (26-24)^2 + (24-24)^2 + (21-24)^2}{24}$$

$$+ \frac{(23-24)^2 + (22-24)^2 + (24-24)^2 + (27-24)^2}{24}$$

$$= \frac{1 + 4 + 0 + 9}{24} + \frac{1 + 4 + 0 + 9}{24}$$

$$= \frac{28}{24} = 1.2$$

χ^2_C has (c - 1) degrees of freedom

where

$c = 4$

$c-1 = 3.$

At the 0.5 level of significance,

$$\chi^2_3 = 7.8.$$

Since the calculated χ^2_C of 1.2 is less than the table χ^2_C of 7.8, we conclude that the difference between samples on different transects is not significant.

The interaction between transect location and sample time (before and after) is denoted by $\chi^2_{I,(r-1)(c-1)}$ and is computed by subtraction. The value is

$$\chi^2_{I,(r-1)(c-1)} = \chi^2_T - \chi^2_R - \chi^2_C = 17 - 12 - 1.2 = \chi^2_{I,3} = 3.8.$$

The tabular chi-square value with three degrees of freedom is $\chi^2_3 = 7.8$. Since $\chi^2_{I,3} < \chi^2_3$, it is concluded that there is no interaction between transect location and sampling period, i.e., there was a uniform reduction in biological forms from transect to transect from the before to after implementation period.

If this were a real situation, we might conclude, based upon the statistical tests, that the number of fish in the area changed after construction of the power plant. From the data, we can see that the number declined so this would be a detrimental effect. There was not a significant difference between transects so the decline was apparently widespread and not just limited to the plume area. The hot water could have reduced the food supply in the general area so fewer fish were supported or there may be other reasons for the decline in fish population. The information resulting from the chemical, benthic, and other sampling programs is essential in answering questions of this type.

The Factorial Arrangement: Analysis of Covariance (Parametric)

The analysis of covariance, combining regression analysis and analysis of variance, is used in this paper to determine the effect of an independent covariate (X), such as temperature, upon a response factor (R), such as biomass of invertebrates per unit of area sampled. This is done by adjusting the sums of squares and mean sums (from analysis of variance) to a common value of the covariate. If, for example, the variability in benthos weight between transects is eliminated when the values are adjusted to a common temperature, one would assume that temperature was a primary factor in causing the variation between transects.

In order for this test to be valid, one must assume that the covariates are not affected by the response factors. For measurements of fish and most benthos, this assumption is probably not violated since it is doubtful that the fish or benthos will materially affect D.O. concentration, stream velocity, temperature, pH, nutrients, or pesticides. It is possible, though, that periphyton, phytoplankton, and aquatic macrophytes do affect D.O. and nutrient concentrations. In this latter case, extreme care must be used in interpreting results because of this dependency. If the response measured (benthos) is affected by the covariate (temperature) and not vice versa, then that covariate can be used without unduly compromising the interpretations.

Biological data may be collected at widely spaced time intervals in comparison to the physical and chemical data. Therefore, some decision must be made by the principal investigator concerning what value to use as a covariate. For example, with temperature, it could be the mean, median, maximum, minimum, or a weighted value per time period. In some cases, more than one of these could be used to determine whether maximum temperature had more effect than mean temperature, or median temperature, etc.

In order to demonstrate analysis of covariance, an example problem is presented which is an extension of the analysis of variance (parametric) problem starting on page The response factor is still the weight of invertebrates per area of sample. The covariate is temperature and we assume the same situation illustrated in Figure 3 (page 52). Good texts, such as Snedecor and Cochran (45), Steele and Torrie (39) or Winer (44) describe this test in detail.

TABLE A-8

SAMPLE PROBLEM RAW DATA SHEET (TEMPERATURE)

Transect \ Width	Block 1 - Spring Const. Time	1 (0.1 W)	2 (0.3 W)	3 (0.7 W)	4 (0.9 W)	T_{sum}	Block 2 - Summer Const. Time	1	2	3	4	T_{sum}	Grand Sums	
1 (above)	1 (before)	50,51 / 101	50,50 / 100	49,51 / 100	49,50 / 99		1	65,64 / 129	65,65 / 130	64,65 / 129	65,65 / 130			
	2 (after)	51,51 / 102	50,49 / 99	50,50 / 100	51,50 / 101	802	2	64,64 / 128	65,64 / 129	64,65 / 129	65,65 / 130	1034	1836	
2 (below)	1	51,50 / 101	51,51 / 102	50,50 / 100	49,50 / 99		1	64,64 / 128	64,65 / 129	65,63 / 128	65,65 / 130			
	2	71,72 / 143	68,67 / 135	51,50 / 101	50,50 / 100	881	2	84,84 / 168	83,82 / 165	65,65 / 130	64,65 / 129	1107	1988	
3 (0.5 ΔT)	1	50,51 / 101	51,51 / 102	52,50 / 102	49,50 / 99		1	65,65 / 130	64,65 / 129	66,65 / 131	65,64 / 129			
	2	61,62 / 123	60,61 / 121	51,50 / 101	50,49 / 99	848	2	75,74 / 149	73,74 / 147	65,65 / 130	65,64 / 129	1074	1922	
4 (0.1 ΔT)	1	49,49 / 98	50,50 / 100	50,51 / 101	51,51 / 102		1	65,64 / 129	65,65 / 130	65,65 / 130	64,66 / 130			
	2	52,53 / 105	53,52 / 105	50,50 / 100	49,49 / 98	809	2	67,66 / 133	67,67 / 134	65,64 / 129	64,64 / 128	1043	1852	
CW Sum		401	404	403	399	1607		516	518	518	519	2071	3678	
		473	460	402	398	1733		578	575	518	516	2187	3920	
W Sum		874	864	805	797	3340		1094	1093	1036	1035	4258	1968 1957 1841 1832	7598

TABLE A-9

SAMPLE PROBLEM INTERACTION TABLE (TEMPERATURE)

Transect	Time	0.1 W	0.3 W	0.7 W	0.9 W	Total
T_1	C_1	101+129= 230	230	229	229	918
	C_2	230	228	229	231	918
TOTAL		460	458	458	460	1836
T_2	C_1	101+128= 229	231	228	229	917
	C_2	311	300	231	229	1071
TOTAL		540	531	459	458	1988
T_3	C_1	231	231	233	228	923
	C_2	272	268	231	228	999
TOTAL		503	499	464	456	1922
T_4	C_1	227	230	231	232	920
	C_2	238	239	229	226	932
TOTAL		465	469	460	458	1852
TOTAL	C_1W	917	922	921	918	3678
TOTAL	C_2W	1051	1035	920	914	3920
TOTAL	GW	1968	1957	1841	1832	
						7598

The data for the covariate are arranged in Tables A-8 and A-9 in exactly the same manner as were the invertebrate data in Tables A-2 and A-4. From Tables A-2 and A-8, we see that in block 1, width 1, transect 1, construction time 1, the numbers of invertebrates collected per sample area were 6 and 10. Temperatures at the corresponding locations and times were 50 and 51 degrees, respectively.

All sums of squares are computed for the covariate in the same manner as they were computed for the response factors in the analysis of variance. Equations for the calculations have already been given in the analysis of variance section. In addition, the cross-products must be computed for use in computing adjusted factor sums of squares.

To separate the sums of squares, those for the response factor (invertebrates) will be subscripted by "R," those for the covariate by "X," and those for cross-products by "RX." Response sums of squares are taken from the analysis of variance section and the others are calculated as follows. Numbers are taken from Tables A-2, A-4, A-8, and A-9.

$(G_{Tot})^2/N$ = GS_R for response (invertebrates)

= GS_X for covariate (temperature)

= GS_{RX} for cross-products.

$$GS_R = \frac{1165^2}{128} = 10,603$$

$$GS_X = \frac{7598^2}{128} = 451,013$$

$$GS_{RX} = \frac{(1165)(7598)}{128} = 69,154$$

$$TTSS_R = 1,256 \text{ (from AOV calculations)}$$

$$TTSS_X = 460,918 - 451,013 = 9905$$

$$TTSP_{RX} = 68,974 - 69,154 = -180$$

$$BSS_R = 279$$

$$BSS_X = \frac{3340^2 + 4258^2}{64} - 451,013 = 6,583$$

$$BSP_{RX} = \frac{(488 \times 3340) + (677 \times 4258)}{64} - 69,154 = 1,355$$

$$TSS_R = 118$$

$$TSS_X = \frac{1836^2 + 1988^2 + 1922^2 + 1852^2}{32} - 451,013 = 457$$

$$TSP_{RX} = \frac{(323 \times 1836)+(251 \times 1988)+(272 \times 1922)+(319 \times 1852)}{32}$$

$$- 69,154 = -229$$

$$WSS_R = 102$$

$$WSS_X = \frac{1968^2 + 1957^2 + 1841^2 + 1832^2}{32} - 451{,}013 = 499$$

$$WSP_{RX} = \frac{(259 \times 1968) + (267 \times 1957) + (315 \times 1841) + (324 \times 1832)}{32}$$

$$-69{,}154 = -225$$

$$CSS_R = 97$$

$$CSS_X = \frac{3678^2 + 3920^2}{64} - 451{,}013 = 457$$

$$CSP_{RX} = \frac{(638 \times 3678) + (527 \times 3920)}{64} - 69{,}154 = -210$$

$$TWSS_R = 117$$

$$TWSS_X = \frac{460^2 + 458^2 + \ldots 226^2}{8} - 457 - 499 - 451{,}013$$

$$= \frac{3{,}619{,}256}{8} - 457 - 499 - 451{,}013 = 438$$

$$TWSP_{RX} = \frac{(84 \times 460) + (80 \times 458) + \ldots (84 \times 226)}{8} - (-229) - (-225)$$

$$- 69{,}154 = \frac{547{,}853}{8} + 229 + 225 - 69{,}154 = -218$$

$$TCSS_R = 125$$

$$TCSS_X = \frac{918^2 + 918^2 + \ldots 932^2}{16} - 457 - 457 - 451{,}013$$

$$= \frac{7{,}238{,}332}{16} - 457 - 457 - 451{,}013 = 469$$

$$TCSP_{RX} = \frac{(162 \times 918) + (161 \times 918) + \ldots (160 \times 932)}{16} - (-229) - (-210)$$

$$-69{,}154 = \frac{1{,}095{,}579}{16} + 229 + 210 - 69{,}154 = -241$$

$$WCSS_R = 108$$

$$WCSS_X = \frac{917^2 + 922^2 + \ldots 914^2}{16} - 457 - 499 - 451{,}013$$

$$= \frac{7{,}239{,}560}{16} - 457 - 499 - 451{,}013 = 504$$

$$WCSP_{RX} = (160 \times 917) + (160 \times 922) + \ldots (163 \times 914) - (-225)$$

$$- (-210) - 69{,}154 = \frac{1{,}095{,}771}{16} + 225 + 210 - 69{,}154 = -233$$

$$TWCSS_R = 101$$

$$TWCSS_X = \frac{230^2 + 230^2 + \ldots 226^2}{4} - 457 - 457 - 499 - 438 - 469 - 504$$

$$- 451{,}013$$

$$= \frac{1{,}817{,}162}{4} - 457 - \ldots 451{,}013 = 454$$

$$TWCSP_{RX} = \frac{(41 \times 230) + (43 \times 230) + \ldots (42 \times 226)}{16} - (-229) - (-225)$$

$$- (-210) - (-218) - (-241) - (-233) - 69{,}154 = \frac{270{,}415}{4} + 229$$

$$+ 225 + 210 + 218 + 241 + 233 - 69{,}154 = -194$$

$$EESS_R = 80$$

$$EESS_X = \frac{101^2 + 100^2 + \ldots 128^2}{2} - 451{,}013 - 6{,}583 - 457 - 499 - 457$$

$$- 438 - 469 - 504 - 454 = \frac{921{,}786}{2} - 451{,}013 - 6{,}583 - 457 - 499$$

$$- 457 - 438 - 469 - 504 - 454 = 19$$

$$EESP_{RX} = \frac{(16 \times 101) + (17 \times 100) + \ldots (25 \times 128)}{2} - 69{,}154 - 1355$$

$$- (-229) - (-225) - (-210) - (-218) - (-241) - (-233) - (-194)$$

$$= \frac{137{,}940}{2} - 69{,}154 - 1{,}355 + 229 + 225 + 210 + 218 + 241 + 233$$

$$+ 194 = 11$$

$$SESS_R = 129$$

$$SESS_X = (50^2 + 51^2 + \ldots 64^2) - \frac{(101^2 + 100^2 + \ldots 128^2)}{2}$$

$$= 460{,}918 - \frac{921{,}786}{2} = 25$$

$$SESP_{RX} = [(6 \times 50) + (10 \times 51) + \ldots(13 \times 64)] - \frac{[(41 \times 101) + (41 \times 100) + \ldots(42 \times 128)]}{2}$$

$$= 68{,}974 - \frac{137{,}940}{2} = 4$$

As a check on the computations, the TTSS computed initially can be compared to:

TTSS = BSS + TSS + WSS + CSS + TWSS + TCSS + WCSS + TWCSS + EESS + SESS

$TTSS_R$ = 279 + 118 + 102 + 97 + 117 + 125 + 108 + 101 + 80 + 129 = 1,256

Calculated $TTSS_R$ = 1,256

$TTSS_X$ = 6583 + 457 + 499 + 457 + 439 + 469 + 504 + 454 + 19 + 25 = 9,906

Calculated TTSS = 9,905. Difference is due to round-off in component SS_X's.

$TTSP_{RX}$ = 1355 - 229 - 225 - 210 - 218 - 241 - 233 - 194 + 11 + 4 = -180.

Calculated $TTSP_{RX}$ = -180.

The results of the above calculations are presented in an analysis of covariance table, Table A-10. The sample error term is combined with each of the terms in the top half of the table to obtain the adjusted values in the bottom half.

The sums of the df and of products values are obtained by addition of the sample error term and the factor being considered (transect, width, etc). For example: transect df = 3 and sample error df = 64; 64 + 3 = 67 df for the summed value. Transect X^2 = 457 and sample error X^2 = 25; 457 + 25 = 482. Transect RX = -229 and sample error RX = 4; -229 + 4 = -225, the summed value. This operation is repeated for each term on top to fill in the bottom left side of the table.

The adjusted values on the right side of the table are obtained as follows.

One degree of freedom is lost in the error term for each covariate used so adjusted df always equals df - 1. For example: in transect + sample error, df = 67; 67 - 1 = 66 which is the adjusted df value.

The adjusted sample error sum of squares is obtained by use of the equation $ASESS = SESS_{R^2} - [(SESS_{RX})^2/SESS_{X^2}]$

In this example, the calculation is

$$ASESS = 129 - \frac{4^2}{25} = 128.4.$$

TABLE A-10

SAMPLE PROBLEM ANALYSIS OF COVARIANCE

		Sums of Products			Adjusted Values				
Source	df	X^2	RX	R^2	df	SS	MS	F'	Tabular F*
Total	127	9905	-180	1256					
Block	1	6583	1355	279					
Transect	3	457	-229	118					
Width	3	499	-225	102					
Construction Time	1	457	-210	97					
TW	9	438	-218	117					
TC	3	469	-241	125					
WC	3	504	-233	108					
TWC	9	454	-194	101					
Experimental Error	31	19	11	80					
Sample Error	64	25	4	129	63	128.4	2.04**		
Transect + Sample Error	67	482	-225	247	66	142.0			
DIFFERENCE					3	13.6	4.53	2.2	3.3

Continued

TABLE A-10 (CONT.)

SAMPLE PROBLEM ANALYSIS OF COVARIANCE

Source	df	Sums of Products X^2	RX	R^2	Adjusted Values df	SS	MS	F'	Tabular F*
Width + Sample Error	67	524	-221	231	66	137.8			
DIFFERENCE					3	9.4	3.13	1.5	3.3
Construction Time + Sample Error	65	482	-206	226	64	138.0			
DIFFERENCE					1	9.6	9.60	4.7	5.3
TW + Sample Error	73	463	-214	246	72	147.1			
DIFFERENCE					9	18.7	2.08	1.0	2.3
TC + Sample Error	67	494	-238	254	66	139.3			
DIFFERENCE					3	10.9	3.63	1.8	3.3
WC + Sample Error	67	529	-229	237	66	137.9			
DIFFERENCE					3	9.5	3.17	1.6	3.3
TWC + Sample Error	73	479	-190	230	72	154.6			
DIFFERENCE					9	26.2	2.91	1.4	2.3

* Tabular F ratios were selected at the 2.5% level of significance to better illustrate the problem.

** EMS = SEMS from Table A-6.

The adjusted transect + sample error sum of squares is obtained by use of the following equation:

$$ATSS + (SESS_{R^2} + TSS_{R^2}) - \left[\frac{(SESS_{RX} + TSS_{RX})^2}{SESS_{X^2} + TSS_{X^2}}\right]$$

In this example, it is:

$$ATSS = (129 + 118) \left[\frac{(4 - 229)^2}{25 + 457}\right] = 141.97 \text{ rounded to } 142.0.$$

Adjusted width + sample error sum of squares is:

$$AWSS = (SESS_{R^2} + WSS_{R^2}) - \left[\frac{(SESS_{RX} + WSS_{RX})^2}{SESS_{X^2} + WSS_{X^2}}\right]$$

$$AWSS = 129 + 102 - \left[\frac{(4 - 225)^2}{25 + 499}\right] = 137.8.$$

The adjusted sums of squares for sample error and construction time, TW, TC, WC, and TWC are calculated in a like manner.

The adjusted df and adjusted sums of squares for the treatment (i.e., transect, width, etc.) + sample error are then used to calculate the adjusted values of degrees of freedom, sums of squares and mean squares for the treatment. Treatment adjusted values for df are obtained by subtracting sample error df from the treatment + sample error df value being considered. For example: adjusted df for transect = 66 - 63 = 3; for width it is 66 - 63 = 3; for construction time, it is 64 - 63 = 1, etc.

Treatment adjusted values for SS are obtained in the same manner. For example: treatment adjusted SS for transect = 142.0 - 128.4 = 13.6. The rest are done in the same way.

Adjusted means squares are obtained by the following equation:

$$\text{Adjusted MS} = \frac{\text{Adjusted SS}}{\text{Adjusted df}}$$

For example: adjusted MS for transect = $\frac{13.6}{3}$ = 4.53.

The calculated F value (F') is obtained by dividing each adjusted MS by the sample error MS. For example: F' for transect = $\frac{4.53}{2.04}$ = 2.2.

The tabular F value is taken from a standard table of F ratios using the adjusted sampling error and adjusted difference degrees of freedom. For example, the F value for transect is obtained for 63 and 3 degrees of freedom and the value is 3.3 at the 2.5 percent level of significance. The 2.5 percent level was chosen arbitrarily because it best illustrated the point of the example problem.

If the calculated F' value in Table A-10 exceeds the tabular value we conclude that the variation in weight of invertebrates per unit area of sample is significant for that factor (transect, width, etc.) even after the data have been adjusted to a common value for temperature. In this case, this does not happen since the tabular F is always greater than the calculated F'. So, we conclude that the differences in benthos are not significant after the effect of temperature has been eliminated.

In the analysis of variance problem, page 61, the effects of transect, width, time, and all the combinations were significant. Now, when the covariate (temperature) effect is eliminated by adjustment to a common value, the effects of transect, width, time, and the combinations are not significant. From this, we conclude that the variation in invertebrate weight per unit area sampled is due at least in part to the variation in temperature. Since the data indicate that invertebrates were reduced in the hottest areas, we conclude that the increase in temperature due to the power plant is detrimental to the system.

This same analysis can be used for all the other possible covariates such as D.O., stream velocity, etc. This will aid in separating out effects of these other factors. Temperature is a strong influence in this case, but the other factors could also be important. For example, velocity changes due to intake or outfall design may affect benthic organisms. This will show up if velocity is used as a covariate. Since a computer is needed for this analysis, all the probable covariates should be run at the same time to give a broader picture.

Factorial Arrangement: Analysis of Covariance (Nonparametric)

Whenever the data exhibit extreme non-normality or are basically a count, a nonparametric analysis is used. In this analysis, the numerical values for response and the covariate are ranked and an ordinary linear regression is performed on the ranked values. The output consists of adjusted responses. These adjusted responses are then analyzed by analysis of variance. This method of analysis is presented in detail in the paper by Quade (43).

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1 Accession Number	2 Subject Field & Group	
	010	**SELECTED WATER RESOURCES ABSTRACTS** INPUT TRANSACTION FORM

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6 *Title*

GUIDELINES: BIOLOGICAL SURVEYS AT PROPOSED HEAT DISCHARGE SITES,

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27 *Abstract*

A quantitative approach is presented for the biological portion of thermal discharge siting surveys and discharge monitoring. Three types of studies are covered: Type I is a very general study of the aquatic system and the pertinent literature; Type II is a comprehensive pre-operational study designed to supply data for management decisions on power plant siting and data to serve as baseline for possible future comparison; and Type III is a post-operational continuation of Type II to detect possible effects if a thermal discharge to a natural water body is allowed.

Two methods are recommended for location of sampling stations by use of a grid system based on planned outfall design. Sample collection and handling methods and frequency of sampling are suggested for fish, macroinvertebrates, plankton, periphyton and aquatic macrophytes.

Methods of data handling recommended include diversity and redundancy indices and a combination of the two into one value for a test for dispersion. A scale of importance is suggested for organisms of special value in either an economic or ecologic sense. For statistical analysis of the data, four appropriate methods are recommended and sample problems are provided to illustrate data handling and conclusions to be drawn from the tests.
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