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DEVELOPMENT OF AQUACULTURE SYSTEMS

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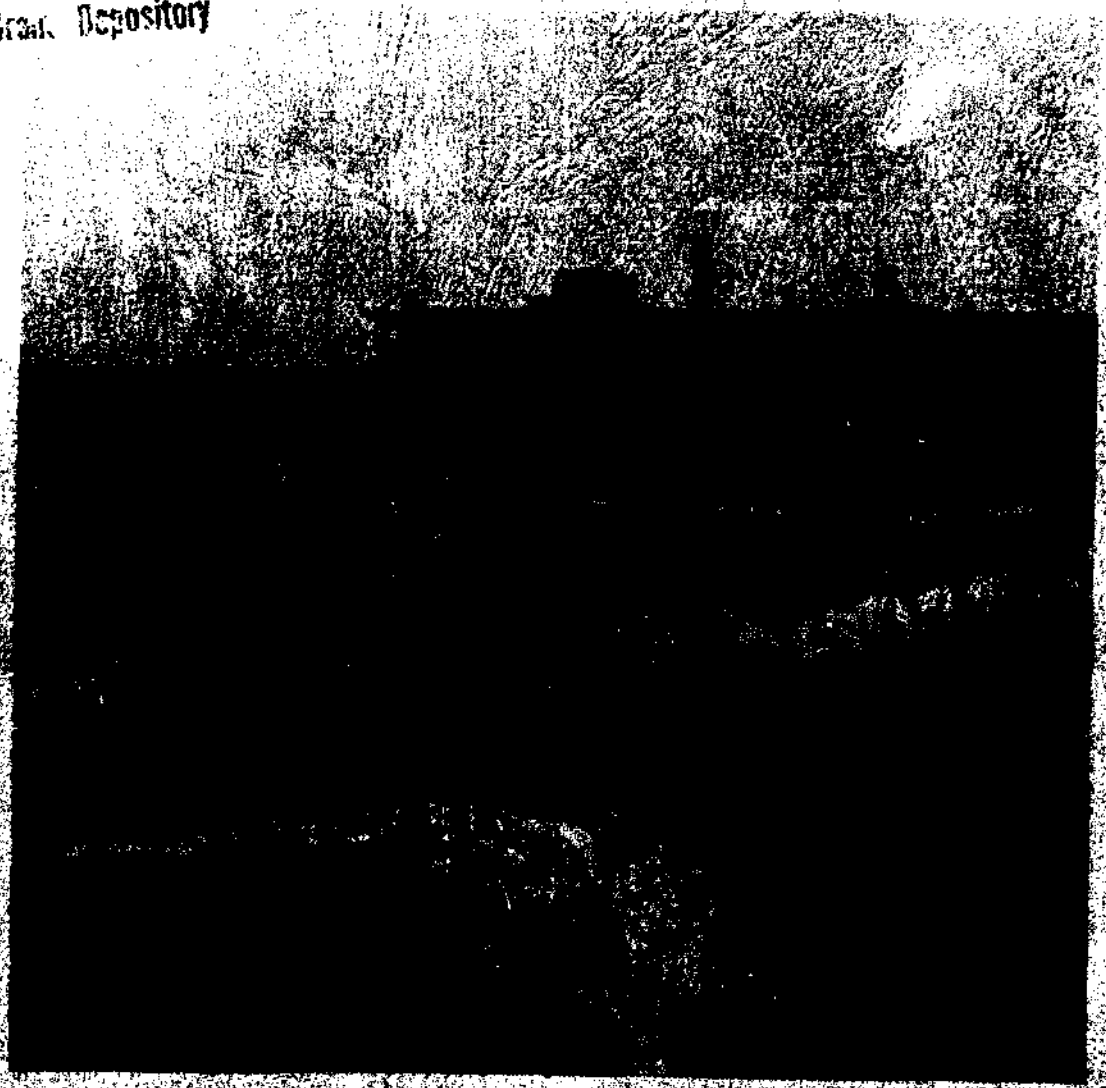

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DEVELOPMENT of AQUACULTURE SYSTEMS

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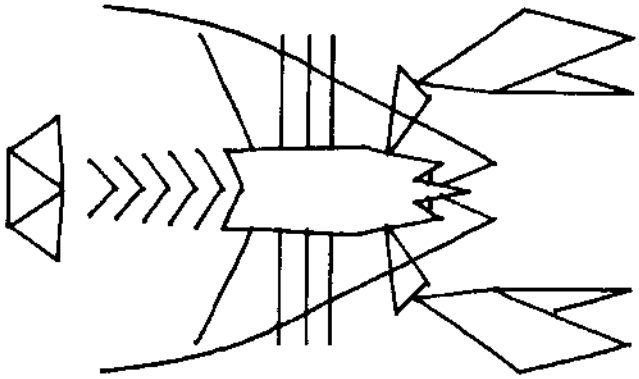

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INTRODUCTION

This report covers the period 1971-1976 and, regrettably, was prepared in the absence of Dr. Robert Shleser, the Project Leader for the first four years (1971-75). Too, a number of persons who played major roles in the early development of the research no longer are with the program. Notable among these are Dr. M.L. Tracey (genetics), Dr. N. Gravitz (water quality), and Mr. A.M. Schuur (program manager). If our perspective in the absence of these original project members fails to identify accomplishments known to them, it is a loss for which we can only offer our apologies.

Initially, this program ranged broadly in its examination of likely candidates for useful aquaculture. Such widely different organisms as Tasmanian freshwater crayfish, grass carp, Asian prawns, other prawns, various crabs, several mollusks and lobsters were explored. Soon, however, our research focused on lobsters (both Homarus americanus and H. gammarus). Earlier studies, largely derived from work at the Massachusetts State Lobster Hatchery, had shown that lobsters could be grown from eggs to adults under confined laboratory conditions. Lobsters seemed to offer a number of advantages not possessed by all crustaceans. Among these were the short, free-swimming larval stages, the apparent hardiness of the larvae and subsequent stages, and the potentially high economic value of the adult animals. We hoped that intensive study of one animal by an interdisciplinary team would yield scientific data and a technology that might serve, in the broad sense, as a general model for crustacean aquaculture. The primary question

posed was, is an economically viable commercial culture of the lobster possible? That single query has guided our research through the five-year period. We are not yet ready to offer an affirmative answer, although that time is near at hand.

Our work fell into a series of definable areas and questions, and this report is written around them. We feel we have mastered larval rearing and that the technology we have developed is fully transferrable to commercial operations. Our nutrition program now has developed artificial feeds that promote growth nearly as well as "natural" foods, and these feeds are ones that can be adapted to mass production by the animal food industry. Also, we are well on our way toward a full understanding of the basic nutritional requirements of lobsters. We have examined their genetics and have shown that heritable variations in natural populations provide the potential for the selection of superior, fast-growing strains. Too, we have developed techniques for "genetic tagging" that can be used to study the effects of the planting of hatchery reared young lobsters or other crustaceans in the field. This is a signal accomplishment, since no device for tagging crustaceans for long term study had previously been available. We have successfully mated lobsters in the laboratory and have grown the offspring. Our matings have produced hybrid offspring between American and European lobsters, and this offers exciting possibilities for the improvement of growth and efficiency rates through "hybrid vigor." We have identified, isolated and learned to control, through our microbiology/ pathology research, a number of potentially devastating diseases of

lobsters and have learned to diagnose system and nutritional deficiencies through pathological analyses of lobsters. Through our systems analyses and economics program, we can now project costs of lobster production with a high degree of confidence; the models developed by that program have guided us in selecting critical areas for further research. We have studied water quality and can now define the environmental requirements of all stages in lobster production; the program has also made fundamental contributions to an understanding of seawater chemical analyses. We have experimented with flow-through (open), semiclosed (partially recirculating), and closed (fully recirculating) water systems and have explored the use of algae (see Algology) in the cleansing of the effluents of aquaculture systems and in the mass culture of juvenile lobsters.

A number of personnel formerly employed in our program have moved on to other academic institutions and to positions in privately funded aquaculture operations. The training we have afforded has provided them with the basis for their upward movement in their professions. Many students have been formally assisted by our program, some by direct support as Sea Grant Trainees and others by association with, and assistance from, the many researchers in our group. Four graduate theses have been completed based upon work in, or with the assistance of, our program. At present, two Sea Grant Trainees are in the final stages of their Ph.D. theses research and another is in the early stages of her Ph.D. program. A visiting graduate student from France is carrying on her thesis research as part of our genetics program.

Only time will evaluate the degree to which our research, our program, and its findings are truly useful in commercial aquaculture. The basic approach we have developed has set standards and provided the technology presently used by a number of small commercial ventures. We are not yet at a time at which lobsters can be produced at a cost competitive with wild-caught animals, but, as the wild stocks decrease, there will be an inevitable increase in the price. As our knowledge and technology of lobster production increases, there is every indication that these two trends (the rising cost of wild-caught lobsters and the decreasing costs of cultured lobsters) will intersect and reverse their relative costs. We are close to the full domestication of the lobster and to the time when economically viable commercial lobster aquaculture will appear.

We have organized our report so that the reader may first visualize our physical systems and the operations that relate to them. The development of these systems, in part, preceded and, in part, occurred along with the development of our more basic research programs. That is, there has been an evolution of our systems, operations, and support functions that has paralleled the needs of our experimental activities. Following this in our report, we turn to a description of the functions and accomplishments of each of the program areas. This more basic research is organized by discipline. At the end of our report, we have provided brief conclusions, a list of our trainees, theses, and cooperating organizations and individuals, and finally a list of papers published, in press and submitted for publication.

ACKNOWLEDGMENTS

Our program has been assisted by many persons and groups. We owe a deep debt of gratitude to our University for its provisions of personnel, funds, and the facilities of the Bodega Marine Laboratory, and a special debt to the staff of that Laboratory for its many kindnesses. The Legislature of the State of California has been generous in its support of aquaculture and our debt to them is great. The National Sea Grant Program and the University of California Sea Grant College Program both have assisted us immeasurably, and the work reported herein was possible only because of the continuous financial support they provided. We would like to thank each of the Sea Grant-related personnel individually, but their names are too many to list here. We would, however, be remiss if we failed to thank Dr. James Sullivan, our Sea Grant College Program Manager, for his continued support and assistance.

Many individual faculty members of our University have participated in our program, have assisted us, and been vital in providing expertise, encouragement and support. Particular thanks are due to Prof. Bernard Schweigert (UC Davis) and Prof. Harold Olcott (UC Davis) for their many acts of assistance, support, and guidance. We also owe Prof. Duane Brown (UC Davis) a debt of gratitude for his collaboration and assistance in our nutritional studies, and Prof. Roger Garrett (UC Davis) and Prof. George Tchobanoglous (UC Davis) for collaborative and consultative assistance in many engineering and water treatment problems. Prof. Warren Johnston (UC Davis) and Geoff Allen (UC Davis) took part in our

systems analyses and economics work for which we are deeply grateful, and Prof. John West (UC Berkeley) was generous to us in the development of our work in algology. Prof. Mary Lou Pressick (UC Berkeley) participated in our work in genetics and in a preliminary field study of lobsters at Martha's Vineyard. We are grateful to her for her collaboration. Prof. S.W. Wellings (UC Davis) generously offered assistance on matters related to the pathology of lobsters, and Prof. Allen Knight (UC Davis) has collaborated with us in a number of helpful and constructive ways. Finally, we owe a debt of gratitude to Profs. Tom Cahill, Robert Brocksen (both UC Davis) and Malcolm Gordon (UC Los Angeles) for support and advice, and lastly to Dr. Maynard Cummings (UC Davis) and his Sea Grant Marine Advisors for their many favors.

Throughout the five-year program, we have been able to offer assistance to many individuals, agencies, and groups and, in turn, have received assistance, advice and encouragement from them. A list of those with whom we have worked appears under the heading "Cooperating Organizations and Individuals" in our Final Report. We have truly enjoyed working with, and we extend our sincere gratitude to them. We also have appreciated the opportunity to relate to the California Sea Grant Seafood Industry Advisory Committee, and sincerely appreciate the advice and guidance they have offered.

A special tribute is due to Mr. John Hughes of the Massachusetts State Lobster Hatchery. Without his early research, his continued friendly advice and cooperation and his constant encouragement, this program would not have been conceived nor would our progress have been so swift.

OPERATIONS

Central to the investigation of lobster aquaculture are the operations which maintain lobster survival and provide controlled repeatable environments within which the necessary experiments may be executed. Sound experimental control and meaningful comparison of different experiments depend on the availability of large numbers of healthy animals that have been reared under nearly identical conditions. Egg-bearing or "berried" females are obtained from various sources, chiefly and most consistently from Massachusetts coastal waters. Hatching occurs mainly in the winter and early summer with the exact timing dependent on the availability of berried females and experimental requirements.

This section discusses the physical systems and procedures related to the routine production of larval and juvenile lobsters and the maintenance of adults. Also presented here are ordinary support procedures for monitoring water quality, diagnosing sick or dying animals and data analysis provided to the program as a result of our interdisciplinary approach.

Physical Systems

The major considerations for commercial lobster aquaculture will be production output, cost, and animal health. Often, these criteria are antagonistic to one another and optimal results require competent management. Our research has provided future culturists with an array of management techniques to allow flexibility and to improve the potential of their operations.

The most promising culture scheme is the use of elevated water temperature which increases the growth and development rates of lobsters (Hughes and Matthiessen, 1962, Limnol. and Oceanogr. 7:414-421). This increases the production capability of each rearing tank and decreases the cost of feeding and maintaining larvae for long periods of time. Yet, there are inherent disadvantages in this practice, especially in the cost of heated water and the health of the animals. The cost of heating seawater is discussed in the Systems Analyses section, and microbial diseases (enhanced by increased temperatures) are discussed in the Microbiology/Pathology section of this report.

In order to reconcile and lessen these drawbacks, several systems and procedural modifications have been instituted. The cost of heating water may be drastically reduced by recirculating the heated water, rather than disposing of the effluent after a single pass as in the flow-through (open) method. However, continuous recirculation (closed system), especially without biological filtration, would eventually be harmful to the animals due to metabolite buildup. We optimize the advantages of both systems by using a semiclosed or partially re-circulating seawater system for lobster rearing. By the addition of replacement water at a fraction (8-16 per cent) of the complete turn-over rate, the semiclosed system reduces heat loss and ensures the constant elimination of metabolites and maintenance of water quality (salinity, pH, salt balance, etc.). A discussion of the various advantages and disadvantages of these three types of systems (open,

closed and semiclosed), including methods of calculating heat input requirements and water-replacement rates, has been published (21).*

Microbial populations increase as a result of elevated water temperatures. The microorganisms may or may not be directly pathogenic to the lobster, but the microbes are potentially detrimental and should be minimized in the rearing systems. We have found the most efficient method of eliminating viable microorganisms from the culture waters is the use of on-line ultraviolet irradiation. Water from the system outfall tank (sump) is passed through microfilters and ultraviolet sterilizers where bacterial numbers are reduced to less than 20/ml.

Pelagic larval and benthic postlarval and adult stages of the lobster require different holding compartments if they are to be reared effectively. Regardless of the animal habitat, however, the same semi-closed recirculating system with on-line ultraviolet irradiation is used in all of our work. Central to this system is a large sump which receives the fresh seawater, filtered of gross particles and supplied at a metered rate. Again, the amount of fresh seawater is only a fraction of that required by an open system. A standpipe in the sump connected to a drain maintains the proper water level. Generally, the sump is also the site where the seawater is heated to maintain the required temperature. Temperature regulation is accomplished by a thermostat with a sensor either in the sump or, if convenient, in the

*In the text which follows, publications of our program are cited as numbers in parentheses. Those numbers refer to the numbers on the list of Publications at the end of this report.

animal housing unit. The thermostat controls a relay that activates on demand either immersible heating elements or a pump that circulates some of the water to a heat source that can be commercial, epoxy-lined water heaters, heat-exchangers, or solar panels. Often, an airlift pump is used in the sump to prevent thermal stratification; this, or course, also provides for additional oxygenation of the water. From the sump, the heated seawater is pumped to treatment units. (The size of the pump is dependent on the size of the total system.) Treatment is generally a three-stage process. First, the water is passed through a sand and anthracite column filter which acts as a roughing filter and provides some biological treatment of metabolites. Although some metabolites are taken out in the column filter, metabolites are primarily removed through continuous dilution and disposal with the partial seawater replacement. Following the column filter the water is passed through successive (50, 25 and 5μm) disposable resin cartridge filters which remove the remaining particulate matter. Passage through an ultraviolet sterilizer completes the treatment process. After passing through the animal holding unit, the water is returned to the sump for recirculation. Various flow meters and valves are used to monitor and adjust the flow of water at critical points. Some details of this system have been described along with diagrams of the various units (21).

Although the semiclosed system has been used extensively at the Bodega Marine Laboratory, studies conducted at the inland UC Davis campus allowed us to develop totally recirculating (closed system) technology. A closed system required the removal of detritus (unused food and fecal

particles) and dissolved metabolites while maintaining proper salinity, pH, and salt and ionic balances. Several water-treatment systems were designed and evaluated; the most successful combination consisted of a multimedia filter, an illuminated sand bed, and an ultraviolet sterilizer. The multimedia filter consisted of a five compartmented, rectangular chamber with alternating flow diverters which gave an upflow in one compartment followed by a downflow in the next, achieving a columnar flow through each compartment. The first was filled with anthracite topped by a polyurethane foam pad to remove particulate waste. The next two compartments were filled with coarse sand, providing substrate for nitrifying bacteria, which converted toxic ammonia to essentially non-toxic nitrate. The following one contained activated charcoal to remove organics, and the final compartment was an aeration area to restore the dissolved gases. From this filter, the effluent water was pumped into a manifold which sprayed fine streams of the water into artificially illuminated containers of sand. This provided a substrate for the growth and proliferation of macro algae such as Enteromorpha and Ulva, the halophyte Salicornia (pickleweed), and several unidentified diatom and blue-green micro algae species. The plants which covered the surface served to remove nitrogenous wastes. Before returning to the rearing tables the water was "polished" by a disposable cartridge filter and an ultraviolet sterilizer.

Temperature control was afforded through a multichannel heat exchanger coupled to a standard hot water heater or to a freshwater sump cooled by portable immersion chillers. Salinity was maintained through

periodic addition of deionized water. Water changes occurred at approximately one month intervals. With this relatively simple system, ammonia was consistently maintained well below toxic levels.

Two identical systems of the above design were operated simultaneously for two 90-day experimental periods. Each system contained approximately 1140 liters of water and was stocked with 36 juvenile lobsters. One system was filled with natural seawater from the Bodega Marine Laboratory, and the other with artificial seawater prepared from Instant Ocean Mix (Aquarium Systems, Inc., Eastlake, Ohio) and deionized water. The growth of the lobsters in the artificial seawater system was significantly better in the first trial, and slightly better in the second trial over that of the lobsters in natural seawater. No obvious explanation was evident as water quality parameters remained essentially the same for both systems. However, the experiment did prove that artificial seawater is a satisfactory medium for the culture of juvenile lobsters. This has obvious implications for future commercial operations where suitable natural seawater is not available (Gallagher and Brown, 1976, Aquaculture 9:87-90).

Rearing and Management Procedures

As mentioned earlier, the larval stages are pelagic, necessitating a constant flow of seawater sufficiently forceful to maintain the larvae in the water column without battering them against the walls of their container. The rearing tank used for larval production (see Fig. 1) is hydraulically designed to disperse the larvae uniformly in the water column and flow rates are adjusted between 4-8 l/min, depending on the

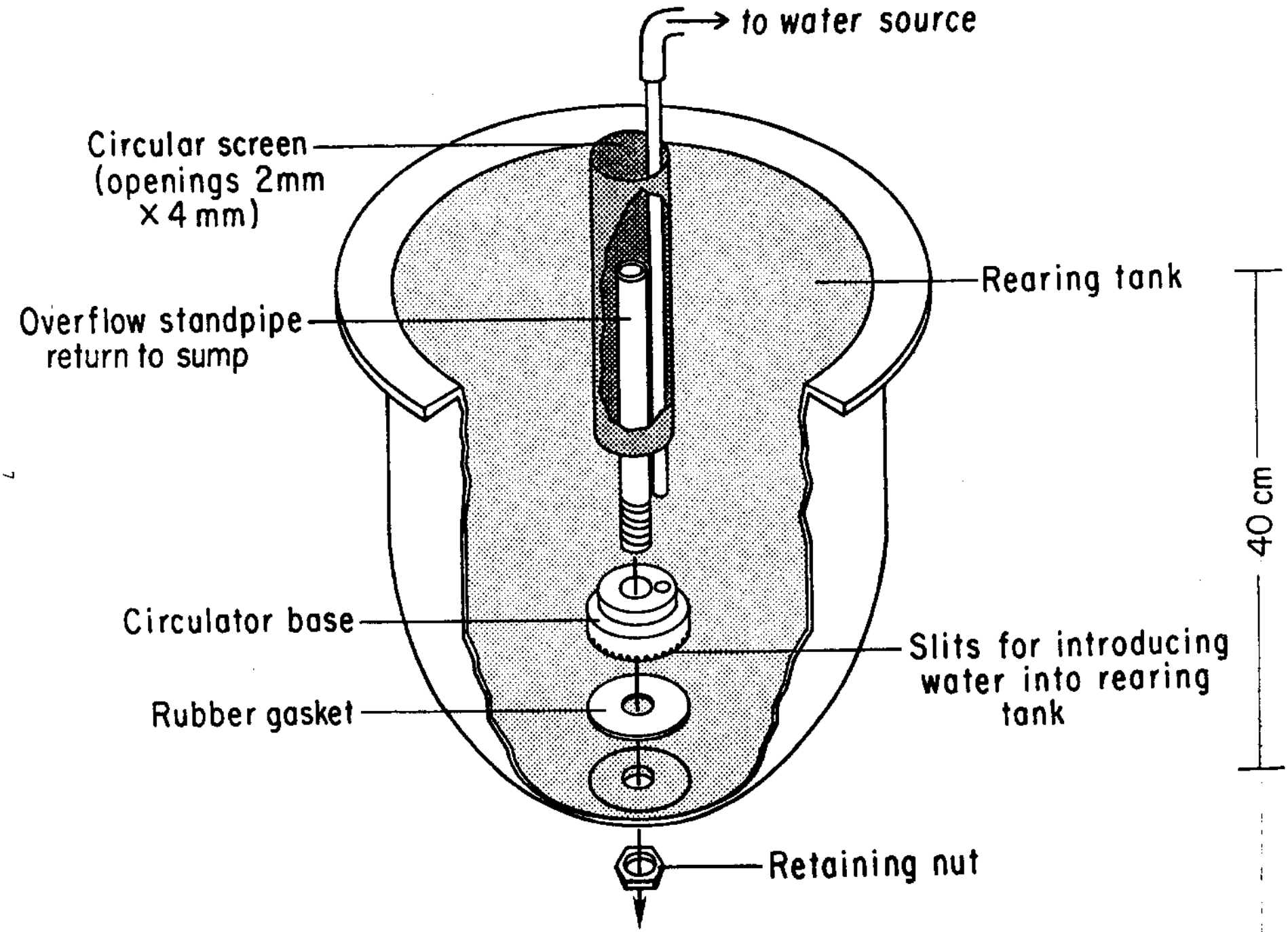

Figure 1. Larval rearing system

number and size of the larvae. If the rearing tank is ineffective or too many larvae are placed in the tank in an attempt to increase production output, mortalities will occur from cannibalism and disease will spread more rapidly. We have modified the original design of the larval-rearing tank to reduce particulate settling and described the hydraulics of the tank (4).

Although the seawater for the larval rearing systems is passed through ultraviolet sterilizers, a large number of microorganisms attach, or adhere, to the surfaces of the animals. In order to eliminate these surface contaminants, we have developed a useful prophylactic method, using the chemotherapeutic staining agent malachite green (19) and determined toxic levels to the larvae (25).

Malachite green has also been found effective for several larval diseases, including those caused by the fungus Lagenidium sp., chitinolytic bacteria, and nonspecific microbial epibionts. The Microbiology/Pathology section of this report deals with these syndromes in greater detail. Antibiotics have been found acceptable for bacterial diseases, but care must be taken that a fungal epidemic is not created by the selective removal of competitive bacteria in the system. The larvae may be made more susceptible to these diseases if certain water quality parameters are substandard. Most important for the sensitive larval stages is the toxic metabolite, ammonia, necessitating the frequent monitoring of this chemical. Larval-rearing practices have been fully discussed in a handbook on hatchery methods (21).

After four molts in the larval stage, lobsters become benthic and are considered postlarval or juveniles. Virtually all of the post-larval studies at the Bodega Marine Laboratory have been carried out using individual habitats (Fig. 2) due to the cannibalistic behavior of the lobsters. They are reared in removable trays which fit into water tables which are typically 121cm x 242cm with an 18cm depth. They are made from plywood coated with fiberglass and have two or three drain holes located on the centerline. The drains are plumbed with an external standpipe at one end of the table and return the water to the sump. Water enters the tables and is directed through distribution pipes around the perimeter. A circular flow of water in the table from the distribution pipes at the outside to the drains at the center provides a scouring action to remove most solid waste products from the table bottom. By adjusting the standpipe at the end of the table, the water level in the table can be exactly fixed.

The animal compartments consist of plexiglass trays with plastic mesh floors which are lowered into the water and rest on the distribution pipes, a few centimeters from the bottom of the table. Typically, four trays are placed in one table for convenient handling and compartment size may vary to allow 72-128 compartments per tray. This system has given excellent service in terms of animal survival, ease of maintenance, and reliability. Animals of almost any size may be accommodated by varying the compartment size, although this system is best suited for the younger juveniles. Trays may be removed at any time to allow cleaning, and all areas can be monitored visually. A complete system (which would

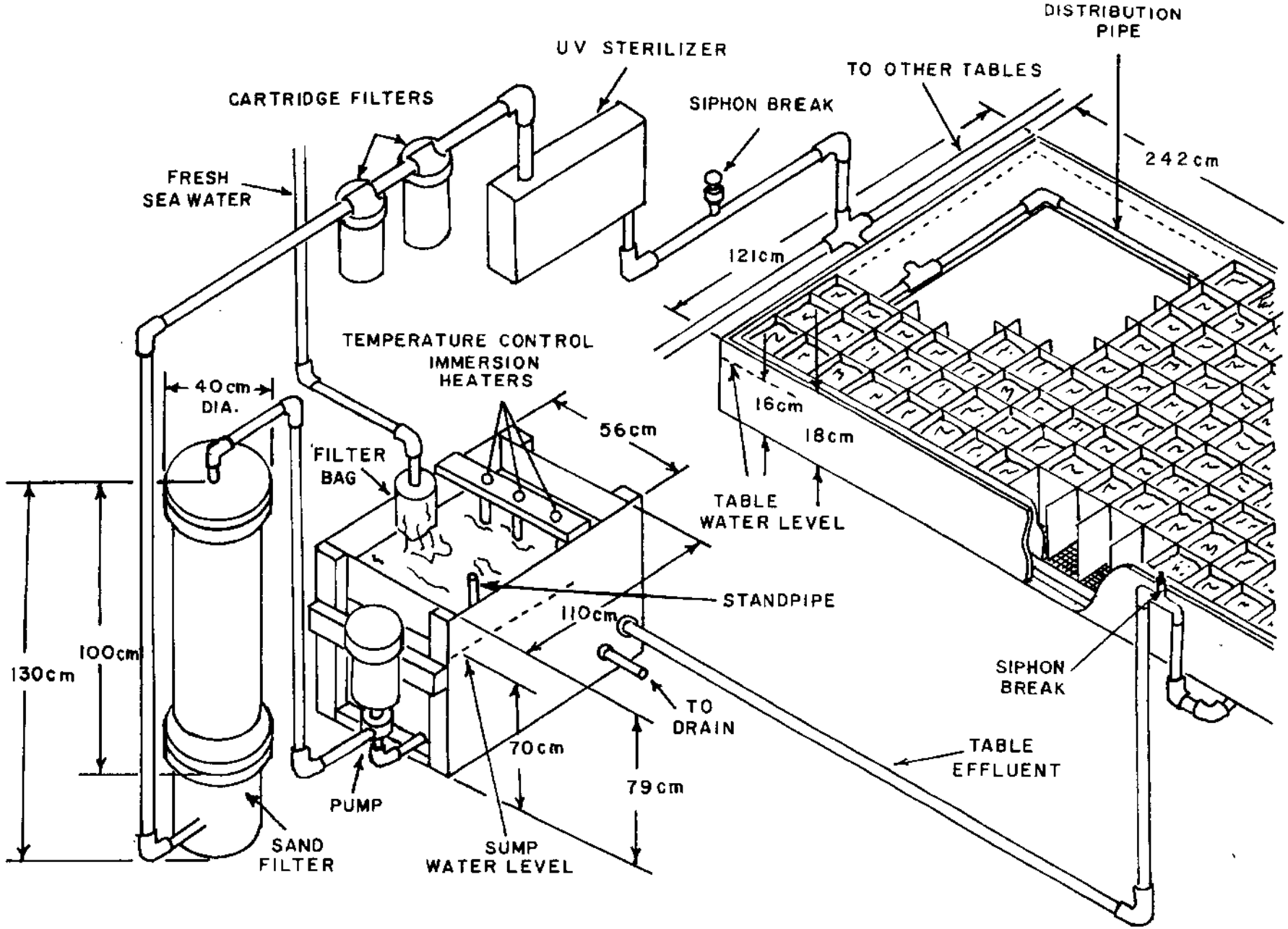

Figure 2. Semiclosed recirculating system with juvenile rearing units

include a siphon break) will halt water loss in case of a pump failure and will recirculate indefinitely if the seawater replacement supply should fail.

Early recirculating systems (a jet of water entered each individual compartment from the top) were difficult to maintain and access to the animals was limited. Our water treatment procedures and physical system designs were added sequentially to improve culture conditions. Although experimental comparisons between the evolving systems were not carried out, our progress can be noted. In an early experiment (6), survival on a diet of live adult brine shrimp was only 85 per cent after three months for one group of test animals, while most of another group were lost due to a system's failure. With the present system, survival on the brine shrimp control diet is generally 100 per cent and system failures leading to animal death are extremely rare.

Adult holding facilities are of two types: shallow troughs and deep tanks (Fig. 3). The preformed fiberglass troughs (36cm x 152cm x 26cm deep) have removable dividers that can be used to make up to 10 compartments per each trough. These units are used primarily for holding males and some potentially valuable broodstock animals. The 72 deep tanks (37cm x 74cm x 49cm deep) have been connected in eight batteries of nine tanks each in order to carry out the broodstock development experiments described in the Genetics section.

Adult lobsters are fed a mixed diet of bottom fish, squid, shrimp, and shellfish as available. Feeding level is regulated by experience so that the animals are not fed to excess but close to satiety. This

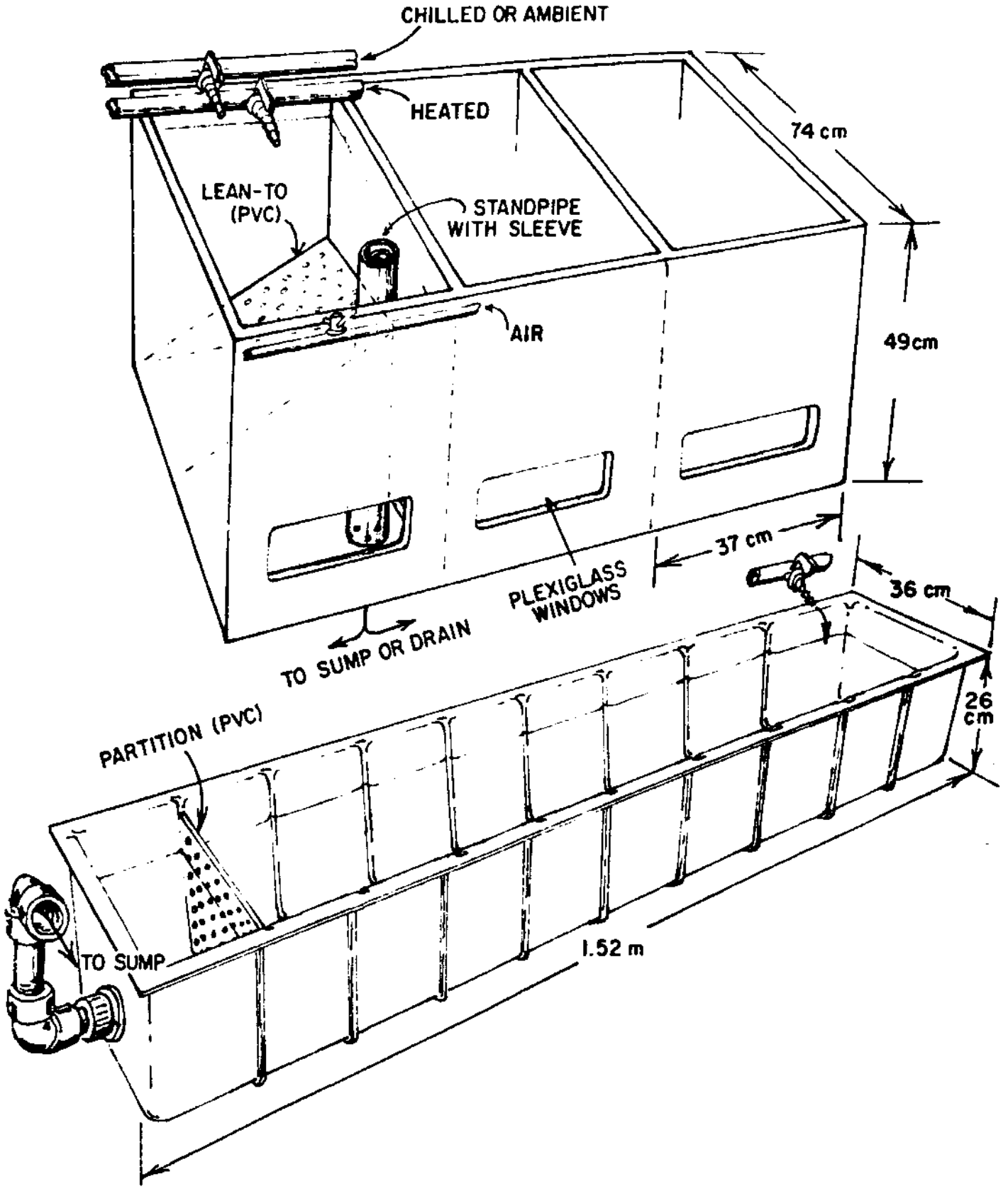

Figure 3. Adult holding

close attention to the proper feeding level minimizes cleaning of adult holding units, generally once a week. Handling of adults is kept to a minimum so that "banding" (placing rubber bands around the claws) of these animals generally is not necessary.

SUPPORT FUNCTIONS

The advantage of an interdisciplinary approach to complex research problems accrues not only from the expertise brought to specific research problems but also from the ability to make routine operational procedures necessary for research, which will be described. While only three of the program areas are featured, other areas perform equally important, though less routine, tasks such as obtaining egg-bearing lobsters and hatching offspring for experimental subjects.

Water Quality

Changes of system variables such as flow rate, animal density, feeding frequency, system turnover rate, etc. (previously described) all have a variable effect upon the water quality. Conversely, a serious adverse change of the water quality can be remedied or offset by appropriate changes of system variables. This interplay requires knowledge of some rather critical components of the water quality. The chemical and the bacteriological components are discussed here as separate entities, but interpretation of both types of monitoring data goes into an understanding of the quality of a water sample. Metabolic wastes and its by-products are important in aquaculture for two reasons. First, these products are often toxic (notably ammonia and nitrite nitrogen) to the cultured species; second, these products must be safely disposed of for general health reasons. In semiclosed culture systems, accumulation of these potentially harmful materials require continuous attention.

The assimilation, utilization, and excretion of nitrogen, and the chemical form of the excreted nitrogen are important variables in both experiments (to be elucidated later), and in systems management. Ammonia-nitrogen is particularly important in management practices since this form is quite toxic at relatively low concentrations. It has been found that about 1300 micrograms per liter is the LC_{50} 96 for small lobsters (for details, see section on Seawater Chemistry). Intake water at the Bodega Marine Laboratory varies from one to 60 micrograms per liter in ammonia-nitrogen. When the ammonia concentration in a system exceeds 100 micrograms per liter, some variable such as makeup rate or backwash frequency must be altered to bring the concentration closer to ambient values. We routinely monitor for inorganic nitrogen (ammonia-ammonium, nitrate, and nitrate) and inorganic phosphate at selected stations. As research needs dictate, more stations and additional assays are added, e.g., total nitrogen, carbon, and phosphorus. The salinity, pH, and dissolved oxygen are also measured as systems needs indicate.

The results of these assays and the bacterial count are excellent indicators of the water quality. Any serious deviation from established norms signals the need for alteration of one or more of the systems variables. To date, some 50,000 separate physical-chemical data points have been measured in our facility. With our automated system, we have provided many outside agencies (private and University) with water quality data and technical assistance.

Live bacteria are ubiquitous; their number at any time or place depend upon growth and death (or removal) rates. These rates, in turn,

depend precisely upon those systems variables which must be known and controlled in the cultivation of lobsters. Thus, together with various chemical assays (most specifically ammonia-nitrogen) routine bacterial live counts indicate the relative water quality of the system. At the Bodega Marine Laboratory, intake water bacterial count varies from 500-2000 organisms per ml (unpublished data). If this water is simply allowed to stand (aerated), the total count will go to about 500,000 per ml while, if a source of nitrogen is added in the form of ammonium, nitrate or protein, the number of bacteria will go to 10 million per ml. Thus, fecal material and unused food left in the system cause extremely high bacterial populations. Systems operated normally (as described) will have less than 20 bacteria per ml. If the bacterial count rises to several hundred per ml and there is no significant change in the concentration of ammonia, then it is likely that the U.V. tubes need to be cleaned. A concurrent rise in bacteria and ammonia indicates that sumps and filters need to be cleaned.

Diagnostics

Animal mortalities occur even in the best of systems and most carefully designed experiments. Often, the proper identification of the cause of death can be indicative of a system malfunction, or that some dietary or other physiological stress is being introduced. A large number of animals have been autopsied. Routinely, the exoskeleton is examined for obvious breaks due to trauma or bacterial erosion. Next, missing appendages are noted and whether good scar tissue seals the wound. The branchial chamber and the gills are examined for discoloration and

melanization. Gills are usually examined microscopically (phase-contrast wet-mount at 400X) to determine if the cellular structure is intact or harbors bacteria. The gross integrity and color of the hepatopancreas is probably the best single, rapid indicator of the animal's health, although phase-contrast wet-mount preparations and stained histological sections of the organ are more discriminatory.

In addition to routine chemical and bacteriological tests, all systems are checked for variation in temperature, water flow (motor failure or clogged lines), molted and dead animals. These checks are made at least three times per day. Most systems are disinfected prior to new experiments and sand-charcoal filters are backflushed every seven to 10 days.

Quantitative Analysis

Quantitative methods are employed to apply current knowledge of lobster growth and metabolism to the design of experiments and to analyze data from experiments.

Questions regarding physical system and statistical design can be answered, based on knowledge of lobster growth and metabolism prior to experimentation. Determination of the minimum-size container each animal must have so that the effect of limited space on growth rates does not confound the results of an experiment is a good example of use of the current model to design experimental systems. Another is the determination of flow rates required, based on current estimates of metabolite production. In order to design an experiment so that statistically significant differences can be determined, a preliminary

estimate of the expected results is useful. These estimates are often made by using current models of growth or metabolism.

Interpretation of the results of experiments performed in our laboratory often requires statistical analyses of the data. To perform the required analyses, we have designed computer programs which store data on magnetic tape and perform different kinds of analyses. Data are stored on tape in terms of a code identifying each animal (e.g., treatment, location, and family), carapace lengths with molt times, initial and final weights, carapace lengths, and date of death. The data can be stored, updated, and edited at any time during the experiment. The various types of analyses performed typically include carapace length-time regressions and plots, survival-time plots, length-weight regressions and plots, biomass-time plots, and analyses of variances in weight or carapace length. These analyses apply to data from growth experiments. Capabilities of storing and analyzing other types of data are programmed as the need for them arises.

Introduction

The primary purpose of our project has been to develop the science and technology necessary for commercially viable lobster aquaculture and demonstrate its feasibility. Over the past five years, the nature of the work performed to accomplish these ends has evolved from making lobster culture biologically possible to making it economically viable. Currently, lobsters can be grown routinely at high growth rates and low mortality, but the cost of culture is not yet competitive with ex-vessel prices in the fishery.

The aquaculture work at our laboratory has focused on defining the relevant aspects of lobster biology rather than constructing large scale commercial culture facilities. As discussed in the previous section, our work on the development of physical systems has been oriented toward research culture systems, although much of the knowledge and experience gained will be applicable to commercial systems. The results of biological experiments are related to commercial culture through a general mathematical model of lobster aquaculture. These results are discussed in this section, under the applicable programs areas.

The relationships between the various program areas and the necessity of each are best seen from a general viewpoint of intensive or controlled system aquaculture. In intensive aquaculture, all aspects of an organism's environment are provided. The cost of culture is the cost of providing this environment, and the benefit -in terms of market value of the product- is reflected in growth and survival. The animal inter-

acts with its environment in various ways, and the task of biological research is to describe these interactions. The animals' growth and survival are in response to characteristics of the environment such as temperature, space, food type, and general water quality. The animal excretes various toxic metabolites which must be removed from the environment; it also consumes food and oxygen which must be provided.

Each of the program areas is concerned with specific aspects of these relationships and each has significant accomplishments. Work in the Seawater Chemistry program is related to describing effects of the chemistry of system environment on survival and of metabolite excretion on the system environment. This program has defined the toxic level of several nitrogenous metabolites and contributed to an understanding of seawater chemical analysis. The Algology program has concentrated on the use of algae to remove metabolites and provide food. The Microbiology/Pathology program describes pathological effects of the environment and prescribes means of minimizing them. This program has identified, isolated and learned to control a number of potentially devastating diseases of lobster and has learned to diagnose system and nutritional deficiencies through analyses of lobster pathologies. Research in the Nutrition program is oriented toward producing a low cost food with high growth rate, high survival, and low metabolite production. This project has developed artificial rations which promote a growth rate approaching that of natural foods and has defined some of the basic nutritional requirements for lobster. The Genetics program is ultimately concerned with heritable differences among lobsters with respect to performance in the culture system. This program has examined genetic variability

in natural and laboratory populations of lobster and other species of decapod crustaceans using electrophoretic techniques, has estimated the heritability of growth rate, and is investigating reproduction for broodstock development. In addition, this program has developed techniques for genetic tagging that can be used to study the effects of planting of hatchery-reared young lobsters or crustaceans in the field. Work in the Systems Analyses and Economics program is concerned with defining the response of growth and survival to environmental variables and integrating results from all program areas for purposes of evaluation in the economic context of commercial culture costs. This program has quantified the response to temperature and space and has developed a model and analysis techniques for cost projection, system optimization, and research direction. The accomplishments mentioned here are only the major results in each area. Further accomplishments and more detail are presented in the following sections.

SEAWATER CHEMISTRY

In the oceans, the concentration of potentially toxic material is quite low, and physical-chemical parameters such as salinity and pH remain fairly constant. A practical, semiclosed aquaculture facility is likely to experience substantial variations with accumulation of toxic material. The end product of nitrogen metabolism, and very toxic to the lobster, is excreted principally as ammonia. Therefore, the rate of its formation and removal from the growth system becomes critical. Fortunately, in physiologically satisfactory seawater, the ammonia is converted to the ammonium ion:

$$NH_3 + H_3O^+ \rightleftharpoons NH_4^+ + H_2O$$

to the extent of nearly 97 per cent. The ammonium ion is not toxic, but even with the equilibrium strongly favoring the ionic form ammonia will accumulate. Under ideal circumstances, the ammonia will also be converted to nitrite and nitrate by bacterial nitrification.

That concentration which will kill 50 per cent of tested 1-gram lobsters in 96 hours (LC_{50} 96) is 1200 micrograms of ammonia-nitrogen per liter, and 1400 micrograms for 3-gram animals (unpublished data). Similar assays with nitrite and nitrate showed these species of nitrogen to be nontoxic at concentrations of 100,000 µg/l and 500,000 µg/l respectively. The 1200 to 1400 micrograms per liter represents the acute toxicity, however, and animals that survived the toxicity test and were allowed to recover in running ambient seawater were found to be sensitive to 200 micrograms per liter when re-exposed to ammonia (unpublished data). When animals are exposed to ammonia (even sublethal concentrations), the most obvious effect is the gross appearance of the hepatopancreas. This

organ in normal, unexposed animals is quite firm, easily removed intact from the body cavity, and the color is emerald green with some brown pigment in the tubules of the gland. Animals exposed to ammonia had a soft hepatopancreas which was necrotic and difficult to remove. The brown pigment was usually missing and, at high concentrations of ammonia, even the green pigment was gone. The chronic concentration of ammonia toxic to small lobsters is not known, but no ill effects have been noted during fairly long (more than 96 hours) exposure to 100 micrograms per liter.

To accurately assay for the very low levels of ammonia found in the Bodega Marine Laboratory intake water, the standard procedures had to be modified and, in order to do so frequently (twice a week), the process had to be automated. While the necessary changes and tests were being made, it was found that a substance believed to be indophenol was produced by exposure of the reagents to light. This substance gives a variable and erroneous value to the ammonia assay (18).

Water samples do have some bacteria; even when the samples are stored in the refrigerator, there is some conversion of the inorganic substances (nitrogen and phosphorus) into organic material, giving erroneous readings after a few days. It has been found (unpublished data) that prefiltration (0.45µm filter) and addition of chloroform preserves the sample.

Seawater is an extremely complex ionic medium. Consequently, the apparent dissociation constant needed to calculate the ratio of ammonia to ammonium had to be experimentally determined. It was found to be $9.65 \pm .03$ at $17^{\circ}C$ (unpublished data). This value agrees closely with one calculated on theoretical grounds.

ALGOLOGY

Algology can function as a necessary and vital component of an aquaculture program due to several inherent capabilities of algal cells. The first of these is the ability to directly provide fresh, nutritious food for animals such as oysters, clams, scallops, or mussels, or to provide food for live food animals such as rotifers, copepods, and brine shrimp that are fed to carnivorous aquaculture species. Additionally, algae may be used to feed the larval stages of many fish and crustaceans which later become carnivorous. Another, and equally important, function of algal cells is their ability to remove nitrogenous wastes from aqueous media. This provides a means to clean aquaculture effluents for possible reuse in closed or partially closed systems, and/or to meet federal, state or local discharge requirements in flow-through systems.

An obvious combination of these two major capabilities is to produce a useful food product from aquaculture wastewater. The economic considerations are that a commercially valuable shellfish might be produced from the wastewater effluent and, thus, lower the cost of waste treatment and add to the income from the operation.

The broad-range goals of the algology program at the Bodega Bay aquaculture project were to establish a functioning algology facility and to explore the applications of algology to the ongoing lobster rearing study.

The fact that the climate at Bodega Bay is very regular, being cool (50-65^{o}F) year-round, and reasonably sunny (0.35 kcal cm^{2}/day yearly average) allowed for the establishment of an outdoor greenhouse

culture facility with only minimal environmentally controlled space required for the starter cultures. Initial experiments were begun to look at the potential use of phytoplankton culture techniques to remove toxic nitrogenous wastes from the water used for lobster culture.

Several species of phytoplankton, which traditionally have been used successfully to culture shellfish, were grown in media which contained ammonia nitrogen at concentrations that well bracketed those which had been determined by the water quality section to be toxic to juvenile lobsters. The diatom Phaeodactylum tricornutum, the green alga Dunaliella primolecta, and the chrysophyte Isochrysis galbana were used. The results were disappointing from one point of view. The removal rate of ammonia by the algal cells was so low at the toxic concentrations (1.2mg/l) that it was infeasible to use a phytoplankton system to detoxify juvenile lobster culture water for reuse. This information, however, led to the use of a conventional, bacterial gravel bed-type filter to convert the toxic ammonia nitrogen to essentially nontoxic nitrate nitrogen. Culture water was recirculated through this filter until nitrate nitrogen accumulated to a level that would support active phytoplankton growth. Nitrate-laden water was then bled off to a phytoplankton culture unit.

The next step in the development of a phytoplankton wastewater treatment system was to design a physical culture system which would lessen or eliminate problems encountered in traditional wastewater algae culture practices. The problems were (in order of importance): 1) contamination of cultures with "weed" species of algae; 2) instability of culture growth due to fluctuating nutrient levels; and 3) inefficient use

of space because of large, shallow ponds required for light penetration.

With these problems in mind, the following system was designed: The European concept of using tall columns rather than flat ponds was considered and adapted. The advantages of the former are that not only do they take up less space per unit volume, but they allow efficient light penetration, allow for better aeration, have a small surface area exposed to possible outside contamination, and can be constructed from inexpensive materials such as polyethylene liners in rolled fiberglass roofing sheets.

The columns would be hooked up so that the flow must pass through a series of individual units. At the inflow end would be the nitrate-laden, filtered, and UV sterilized effluent water. A constant drip feed of seed algal culture would also be introduced at this end. The idea was to have a high nutrient load and a low algal cell number in the first column(s). As the flow proceeded, the algal cells would proliferate and flow into the next column with a lowered nutrient load and an increased cell count and so on down the line until, in the end column(s), there would be a high algal cell count and a low nutrient level. The decreased nutrient uptake due to low concentration would still be effective overall because of the high cell count. The one-way flow would limit accidental contamination to only downstream units, and the modular design would allow shutdown of part of the system for periodic maintenance. With sufficient testing, enough culture modules could be hooked up to accommodate the highest possible concentration of nutrients coming into the system; thus, the entire system would self-adjust to varying nutrient loads below that level.

The above-described system was in the construction stage when the Holmes ad hoc Sea Grant Review Committee recommended that algology studies at Bodega Bay be terminated and the efforts redirected to areas more closely concerned with crustacean aquaculture. The studies were terminated, and the algology group began to study feeding and culture of larval Dungeness crab.

Some other areas explored by the algology group included the evaluation of tuna cannery wastewater effluent as a nutrient source for phytoplankton culture and possible shellfish aquaculture. The results of this study (37) indicated that, with conventional waste treatment, tuna wastewater could support phytoplankton growth near that of a conventional mineral culture medium.

One of the constraints to lobster aquaculture derives from the aggressive, territorial, and cannibalistic nature of these animals. Visual contacts between lobsters commonly lead to aggressive interactions, and the losers in these combats not infrequently are consumed by the winners. A number of attempts at mass rearing were carried out in the program, one of which involved the culture of juvenile lobsters in nearly opaque "greenwater."

A group of highly cannibalistic 4th-stage lobsters were placed in a single, noncompartmented tank. The culture water was enriched with algae culture nutrients and seeded with a starter culture of Dunaliella primolecta, a green flagellate. The algae bloomed and, for 90 days, the lobsters were maintained in a dense ($1x10^5$ - $1x10^6$ cells/ml) culture of algal cells. Adult brine shrimp (Artemia) were added and maintained at about 1/ml. At the end of the experiment, the lobsters had ex-

perienced something over 30 per cent mortality. The greenwater animals averaged one and a half times larger (carapace length) than their siblings as well.

Although no firm conclusions for the low mortalities and higher growth rate could be made due to the uncontrolled nature of the experiment, there were several possibilities: 1) the turbid conditions masked visual contact between lobsters, 2) the algal cells absorbed or camouflaged the smell of vulnerable newly molted individuals, 3) the animals had well fed live food available all the time, and 4) the algae removed nitrogenous wastes from the culture water. Although these studies have not been completed, mass rearing of young lobsters, with high survival, would offer significant savings in labor, space, facilities, and capital, and would be a boon to commercial lobster aquaculture.

MICROBIOLOGY/PATHOLOGY

Successful cultivation of the lobster was anticipated, in part, from the fact that this animal had been known to be subject to only a few microbial diseases (notably gaffkemia, shell disease, and some parasitic diseases). Furthermore, there were no indications that these animals concentrated or harbored microorganisms or chemicals harmful to man.

The overall objective of this program area is and has been the continuous maintenance of the health of the cultured animals. This general concern has necessitated serious investigation into several areas. Work done may best be presented, not in chronological order, but through some well-defined categories: 1) the development of diagnostic and monitoring procedures, 2) the recognition of new and the re-evaluation of known infectious diseases, and 3) the consideration of noninfectious diseases.

Development of Diagnostic and Monitoring Procedures

Gross and histological anatomical features of adult lobsters have been studied in some detail; yet, there is little published knowledge of such features in larval and juvenile lobsters. One of our early major concerns was to establish the gross anatomical and physiological bases of "healthy" or "normal" larvae and juveniles. This, of course, is a prerequisite for recognizing unhealthy or diseased animals.

A large number of animals (larvae, juveniles, and adults) have been examined. Most of these animals died in standard holding facilities or under specialized research conditions. Some normal animals were sacrificed for control. Several easily recognizable features emerged as indicators of the health of the animal. First was the integrity,

the firmness and color of the exoskeleton. In the healthy animal, a characteristic pigment is deposited in the endocuticle (just under the epicuticular surface layer). While the pigment per se is probably not significant to the health of cultured lobster, its absence signifies a faulty exoskeleton which later will be shown to impair the primary defense mechanism against microbial invasion. Preening, swimming, and normal movement of larvae and young juveniles prevent (in normal animals) accumulation of large numbers of microorganisms on the exoskeleton. However, if the rearing system has an excess of nutrients, the proliferation of microorganisms is so rapid that larvae and sometimes juveniles become totally infested with a variety of microbes.

Color and integrity of gills is a second indicator of the animal's health. These organs in the healthy animal have a clean surface and a well-defined cellular structure. In diseased animals the surface of the gills are often covered with microorganisms and the cellular structure is disturbed or totally missing. As with the exoskeleton, the color of the gills is a good indicator of health or disease. Invasion of any foreign material causes the production of the enzyme polyphenol oxidase in the blood of lobsters which causes hemocytes to become necrotic and form a reddish brown pigment (melanization), which is easily seen in the otherwise opaque gills.

Third, and possibly of the greatest diagnostic value is the hepatopancreas, a gland (midgut gland) occupying a large part of the body cavity. It is an evagination of the midgut enormously expanding the digestive and absorptive surface. This organ consists of a large

number of lobes, each lined with characteristic cells. In normal young lobsters the hepatopancreas is large, firm, and emerald green with many brown rosettes. Almost any pathological condition results in both macro- and microchanges in the hepatopancreas. There may be discoloration, shrinkage, necrosis, and melanization. Changes observed in these various tissues under a variety of disease conditions have been published (32) and form the basis for the routine diagnostic procedure described in a previous section of this report.

As indicated earlier, the number of bacteria in the culture system is one index of water quality. In order for this simple but powerful tool to be useful, the bacteriological characteristic of the input water must be known. It has been found (unpublished data) that, at the Bodega Marine Laboratory, the number of countable bacteria range from 500-3000 per ml. About two-thirds of these are marine forms; the rest come from terrestrial runoff and wind-borne spores. Seasonal upwelling results in demonstrable increase in the microbial count. Potentially pathogenic (to the lobster) bacteria are found in every sample. There is sufficient nutrient in the intake water to support a final crop of 50-500 thousand countable bacteria. No one bacterial form predominates, and nitrogen always has been found to be the limiting nutrient.

A large number of the dead animals died during the molting process or soon after (still soft). In many cases, the shedding process could not be completed because, 1) the appendages were entangled with filamentous algae and bacteria, and 2) the old and new exoskeletons were welded together by sections of melanized material.

Microbial Diseases

With the improved diagnostic capabilities, it became obvious that several new disease entities existed in our facilities and in other rearing facilities. It became our second major task to completely describe the symptoms and the etiology of the diseases and to suggest therapeutic procedures. During this time also, it became evident that some of the previously described diseases had to be re-examined.

New Diseases

Haliphthoros milfordensis is a phycomycetous fungus that invades the tissue of young juvenile lobsters. It is recognizable by the melanization which occurs at the sites of infection, usually the bases of the walking legs and the interior of the branchial chambers. Melanization is the attempt of the lobster to encapsulate or wall off the progress of the fungal mycelium. This results in a brown "scab" at the site of infection with mycelium projecting in different directions and tinged brown from encapsulating melanization. The fungus parasitizes the epithelial layer of the exoskeleton and allows secondary invasion by other microbial agents. Death may also be caused by mechanical impairment of the exoskeleton during molting. The fungus is spread through culture water to other animals by the production of laterally biflagellate zoospores which may exhibit some chitinolytic activity to gain entry into the chitinous exoskeleton. Older juvenile lobsters and adults are not susceptible to this disease, presumably due to the increased thickness of their exoskeletons. The work completed concerning this disease included large scale epidemiology, animal examination,

fungal isolation and description, and the completion of Koch's postulate which involves the inoculation of undiseased animals, contraction of the disease by those animals and reisolation of the fungus from the diseased animals. This work has been published (11) and the investigation of potential treatments was conducted at UC Davis by D. Abrahams and D. Brown with our cooperation.

We reported another phycomycetous fungus as being pathogenic to lobster larvae. This fungus, Lagenidium sp., has been held responsible for diseases occurring on other marine decapod crustaceans, but is particularly severe with lobster larvae. Left untreated, the disease may cause more than 90 per cent larval mortality in two to three days. Characteristically, the fungus consumes the larval tissue entirely, leaving empty exoskeletons to float in the rearing tanks. A system that becomes infected with Lagenidium is most often irretrievable due to the fast growth of the microbe. Like H. milfordensis, it is passed through the culture water by motile zoospores, which, apparently, are capable of penetrating the thin larval integument with a germ tube to provide entry to a variety of marine decapod hosts (20; personal communication, Dr. C. Bland, University of North Carolina, Dr. D. Lightner, University of Arizona, D. Armstrong UC Davis). Again, the thicker exoskeletons of older animals appear to protect them from infection.

Nonspecific microbial epibionts is a name given to a variety of microbes that attach themselves or adhere to the surfaces of lobster eggs, larvae, and young juveniles. The microbes include filamentous and nonfilamentous bacteria, green algae, blue-green algae and stalked protozoans. They are nonpathogenic, yet their presence occludes

respiratory surfaces (egg membranes, larval and juvenile gill membranes) and restricts gaseous exchange which leads to anoxia. The condition is particularly severe during periods of low oxygen tension or during the demanding periods of hatching or molting. Sometimes, filamentous blue-green algae become long enough to entangle larvae and impair molting. Microbial epibionts are wholly dependent on nourishment from the culture water and use the animal surfaces only as attachment or settlement substrate. The dissolved nutrient content in the seawater then is a very influential factor, and poor water quality may be considered an indirect cause of this disease. Microbial epibionts do not penetrate the egg membrane or the larval integument, but may be passed to subsequent developmental stages during hatching and molting by contact with shed exoskeletons. We have reported the occurrence of this disease on lobster eggs and larvae (31). The natural occurrence of this disease on Dungeness crab eggs may be a factor in the decline of crab populations in the San Francisco Bay region (22). From a thorough characterization of the disease on these crab eggs we learned that the most harmful epibionts are nonfilamentous microorganisms that are susceptible to antibiotics (33).

Investigation of potential treatments for these diseases has focused on the chemotherapeutic dye malachite green, the antibiotics streptomycin and penicillin, and ozonation of the culture water. Antibiotics are a specific therapy, selectively eliminating most bacteria. They are particularly useful against the free-living bacterial forms of microbial epibionts that are believed to be the most harmful. Often, however, light or early infections of Lagenidium may be disguised by

microbial epibionts, and the use of antibiotics reduces any bacterial competition that may exist, allowing an opportune situation for the unsusceptible fungus (20). Therefore, a general or broad-spectrum therapeutic is necessary. We have found malachite green to be quite effective against Lagenidium, most microbial epibionts, and chitinolytic bacteria that cause shell disease. We have also determined levels and exposures of malachite green that are toxic to lobster larvae (19, 25). Ultraviolet irradiation of the culture water has proven successful (19) in retarding the influx and growth of microbial populations and has been used extensively in our larval and juvenile rearing systems. We have also investigated the use of ozone as a microbial deterrent. This procedure is similar to ultraviolet irradiation of the seawater except that oxygen is treated and bubbled through the water. Preliminary results have shown that therapeutic levels of ozonated water have no toxic effect on lobster larvae (17). Better understanding of the effects of ozone in seawater are, however, required before this may be suggested as a viable aquaculture treatment.

Further Study of Known Diseases

The most widely studied lobster disease is gaffkemia, a systemic disease caused by the bacterium Aerococcus viridans var. homari. Our treatment of this disease has been to quarantine incoming animals, diagnose the disease from moribund or dead animals, and treat with antibiotics those infected adults that are particularly important to our program. All cases of gaffkemia that we have observed at this laboratory have been from animals recently shipped from the East Coast.

A disease caused by one of the fungi imperfecti, Fusarium spp.

was found and reported by other researchers. We have confirmed their findings and determined species variation between the causative agents from the different sites.

"Shell disease" is the common term for destruction of the exoskeleton by chitinolytic microorganisms. It has often been reported in commercial holding pounds where adult lobsters are confined in high numbers. We have determined that the disease also occurs in larval and juvenile stages where it is responsible for a greater number of mortalities than in adults with thick exoskeletons. It is recognizable by pitting and erosion on the exoskeleton which may lead to secondary invasion by other microorganisms. The chitinolytic agents must first breach the lipid outer layer (or epicuticle) of the exoskeleton in order to reach the chitinous layers. This can happen in a variety of ways such as wounding, through setal or tegumental ducts (which open to the surface), or possibly as a result of lipolytic bacterial symbionts that initially break down the epicuticle for the invasion of chitinolytic bacteria. We have learned that one may increase animal susceptibility to chitinolytic bacteria by feeding synthetic diets. It is felt that these diets lack a component required for adequate epicuticle formation, thus allowing chitin-destroying bacteria easy access to the chitin regions. Although we have not yet determined the nutritional insufficiency, scanning electron micrographs of exoskeletons support this theory (30). We have found that the concentration of bacteria is not important in shell disease and that increased animal susceptibility due to stress is the key factor. Even animals that are wounded can withstand chitinolytic bacteria in the culture water if they are not stressed

and are fed a diet presumably capable of providing a full complement of material for the intermolt repair of the epicuticle. Results of these investigations have been presented (29).

Increased Susceptibility and Noninfectious Diseases

It is evident from the previous considerations that potentially pathogenic microorganisms in the system do not necessarily cause disease. Often, the animals become infected because of sublethal environmental conditions that stress the animals or, in some manner, decrease their resistance. Shell disease is a good example since it is most frequently found on cultured juveniles that are fed inadequate synthetic diets. The link between diet and the epicuticular barrier against infection has already been discussed and many infectious diseases may actually be secondary to conditions of stress. As mentioned earlier (section on development of diagnostic procedures), animals in the process of molting and immediately afterward are extremely sensitive to adverse environmental conditions. While the exoskeleton is soft, the spores of the fungi H. milfordensis and Lagenidium sp. may penetrate more easily. Larvae are, in general, more vulnerable to infectious diseases. This higher susceptibility may be related to the observation that melanization so characteristic of the juvenile defense system is not seen in larvae.

In addition to these stresses are those physical-chemical parameters that left unaltered would eventually kill the animals. These noninfectious diseases may severely stress the animals while still at sublethal levels, greatly increasing their susceptibility to infectious diseases. Dissolved oxygen content of the culture water is one of these

factors, especially for animals whose metabolism is increased by elevated water temperatures. Oxygen tension must be maintained at high levels during molting or when microbial epibionts cover a portion of the respiratory surface. It is well known that the metabolite, ammonia, becomes toxic to lobsters. We have additionally found that even sublethal concentrations of ammonia dramatically alter the hepatopancreas. Although we have not determined the specific mechanism of the alteration, it causes necrosis or breaking down of that gland. High temperatures also have adverse effects on the hepatopancreas. Sublethal destruction of the gland occurs at 27°C for H. americanus, while 21°-24°C are the optimal growth temperatures, and 30°C causes fatalities within a short period of time. The increased metabolism at 27°C causes the lobsters to utilize their own protein which they obtain by slowly destroying the hepatopancreas. This is supported by higher ammonia production levels of lobsters maintained at 27°C.

In addition to results obtained from experiments by this group, much work is done in cooperation with the other groups in our program. For instance, some of the conclusions presented come from a recently completed experiment with the genetics and nutrition groups. The work of the group has substantially increased the probability of commercial lobster aquaculture.

NUTRITION

From the very beginning, development of a cost-effective synthetic lobster diet was seen as one of the project's primary goals. However, it was not until several years later that the magnitude of this central problem was appreciated. Early results, using diets obtained from researchers working with other aquatic animals, were disappointing, as these studies demonstrated the need for a complete nutritional program rather than a relatively simple feed development program. An evaluation of the available literature suggested that much of the effort would have to be directed toward understanding specific nutritional requirements. Much of the previous work on crustacean nutrition, primarily with penaeid shrimp, had concentrated on the development of inexpensive rations made from by-products of marine origin. Definition of nutrient requirements had been largely ignored. As the by-products are complex and tend to be highly variable in quality, little progress had been made in providing guidelines as to what a crustacean diet should contain. Additionally, it was seen that the need for individual compartments in order to prevent cannibalism coupled with the use of heated water to increase growth rates probably mandated the development of intensive systems for lobster aquaculture. As natural food sources will be absent in these intensive, factory-like systems, all nutrient requirements will have to be supplied by the formulated rations. This is a very different set of circumstances than those found in shrimp culture where natural-food organisms in ponds or raceways provide nutrients lacking in the formulated feeds.

Considering these factors, it was decided that definition of basic nutritional parameters for the lobster was the best way to foster the development of economical and practical rations (26). Inexpensive, or least-cost, formulation of rations by the feed industry is dependent on knowledge of three factors: 1) nutrient content of ingredients, 2) digestibility of these ingredients, and 3) nutritional requirements of the animal. A wide variety of feed ingredients have been subjected to chemical analyses, and the industry has available various tables on the nutrient content of these feedstuffs. Based on these tables, the industry can substitute the least expensive ingredient available at any particular time, while maintaining a predetermined nutrient profile by taking into account each species' ability to digest the various ingredients. While nutrient requirements and digestibility values have been worked out for many animals, they have not been defined for crustaceans. Once these factors are known, the aquaculturist, with the help of the feed industry, will be able to formulate least-cost rations. This ability would allow aquaculture to take advantage of the tremendous purchasing power generated by the feed manufacturer's annual use of large volumes of feedstuffs.

We began with virtually nothing known about the nutritional requirements of lobsters. Information on the diet of lobsters living in nature was, and still is, extremely limited. The best source of information at the time came from the work of John Hughes, Director of the Massachusetts State Lobster Hatchery. Over a period of 12 years at the Martha's Vineyard lobster hatchery, he found hatchery lobsters could

be reared if they were provided with a variety of fresh foods. Growth and survival were less than satisfactory when single food items such as shrimp heads, clams, crabs, oysters and fish were used. A combination of these items produced high survival and steady growth. The difficulty of obtaining a specific organism on demand precluded the possibility of carrying out careful feeding experiments using these food sources. There was a need for a single food source which would serve as a standard control diet against which synthetic diets could be evaluated. Live adult brine shrimp, although limiting in some aspects, proved to be suitable.

Lobsters can effectively utilize brine shrimp from hatching through the first three to four months of the juvenile stage. Beyond approximately four months of age, the rate of growth begins to decrease, due to the lobster's requirements for larger food. Survival on the brine shrimp diet is generally close to 100 per cent and growth over the first four months has consistently proven to be superior to any other single food scource examined. The best growth is achieved when the juvenile lobsters are fed a fresh portion of brine shrimp on a daily basis (6). The daily amount fed is five per cent of the animal's body weight; this amount is in excess of what is generally consumed.

The first experiment using formulated rations compared a number of artificial diets which were available from work done with other aquatic animals (7). The most successful of these was a penaeid shrimp diet (FST 24 5/72D Louisiana State University) consisting of 31.5 per cent shrimp meal, 8 per cent fish meal, 2 per cent fish solubles, 3 per cent soybean meal, 19 per cent rice bran, 1 per cent lecithin, 5 per cent

whey, 10 per cent starch, 10 per cent single cell protein, 5 per cent fish oil, 2 per cent vitamin mix, 2.5 per cent kelgin, and 1 per cent calgon. Although this diet was the most promising of the synthetic diets tested, growth was very poor when compared to the brine shrimp control diet. Since available diets were not satisfactory, a complete nutritional program was initiated at the Bodega Marine Laboratory for the lobster.

As the first step, an analysis of brine shrimp was carried out at Davis in order to provide a rough guideline for further diet formulation (12). Later, the capacity for extruded diet manufacture was set up at the Bodega Marine Laboratory. A macaroni-type laboratory extruder (DEMACO 25 lb/hr Lab Press) was installed for the extrusion of experimental crustacean diets. Additionally, a suitable variety of auxiliary equipment for preparation, balances, mixers, etc., and storage of experimental diets, was also procured. Rearing systems were designed and set up for diet evaluation with juvenile lobsters under controlled conditions. These systems are described in the Operations section.

The first experiment using diets manufactured at BML indicated that a fairly well defined diet, E-1, produced a growth rate almost as good as the complex shrimp diet. This E-1 diet consisted of 35 per cent soybean meal, 15 per cent wheat gluten, 25 per cent rice bran, 5.5 per cent corn starch, 15 per cent yeast, and 4.5 per cent lipids. Although these early experiments did little to provide a commercially useful diet (growth was still less than half that achieved with the brine shrimp control diet), the E-1 diet did provide a defined starting point (34).

Using the diet, E-1, the Nutrition group has spent the last several years studying the impact of various nutrients on the growth and survival of juvenile lobsters. This work has been extremely frustrating at times because of the multitude of factors which need to be considered and the lack of any information on these factors in the literature. Quite often we have been unable to demonstrate the effect of a specific class of nutrients because other nutrient deficiencies produced an overall retardation of growth. This type of pioneering diet development proceeds very slowly until a general outline of all the nutrient requirements has been achieved. However, once this stage is reached the further development appears quite simple and dramatic. Fortunately, the work over the past five years has yielded this overall picture of nutrient requirements, and we are, therefore, close to moving on to the final stage of development.

The pattern which has emerged was derived from a number of experiments carried out both at the Bodega Marine Laboratory and on the Davis campus and by Dr. Castell of Canada. Ms. Margie Gallagher, a graduate student under Dr. D. Brown, carried out most of the work on the Davis Campus as well as some of the early work at the Bodega Marine Laboratory. Diet manufacture for the work at both locations was carried out at the Bodega facility.

Protein Requirements

As protein is needed for growth, amino acid requirements and optimum protein levels were examined very early. Work at Davis defined the essential amino acid requirements of the lobster (Gallagher, 1976. Fed.

Proc. 34:880). Using radioisotopic techniques, 10 amino acids were indicated as being essential for juvenile lobsters. These amino acids, valine, leucine, isoleucine, lysine, threonine, phenylalanine, tryptophan, arginine, histidine, and methionine, are the same 10 which have been shown to be essential for other animals. Unfortunately, the quantitative requirements for each of these amino acids is not shown by radioisotopic techniques and have yet to be worked out.

With other animal species, the quantitative requirements for the essential amino acids have been defined by using mixtures of pure amino acids. This approach with aquatic crustaceans is not possible, however, because isolated amino acids are completely soluble in water and would leach out of the diet. One approach to solving this problem is to use a number of proteins which contain differing amounts of each amino acid. After enough different protein sources have been examined, a reliable interpretation as to the probable quantitative amino acid requirements can be made. More recent experiments, for example, indicate that albumin is a much better choice for promoting growth in lobster than casein (Conklin, unpublished data). This probably is a result of an optimal balance of amino acids being present in albumin. This, however, is speculative since little is known about the digestibility of these various proteins.

Since the optimum mixture of proteins needed to satisfy amino acid requirements is yet to be defined, the minimum protein levels required to sustain rapid growth cannot be specified. A reasonable range is between 11 per cent (30) and 42 per cent which is the protein content of brine shrimp (12).

Lipid Requirements

The general inability of arthropods to synthesize cholesterol has been well documented in the literature. The optimum level for cholesterol in the diet for juvenile lobsters has been defined to be approximately 0.5 per cent of the dry weight of the diet (Castell et al., 1975. J. Fish. Res. Board of Canada 32:1431-1435). In addition, Castell and Covey (1976, J. Nutr. 106:1159-1165) have suggested a requirement of unsaturated fatty acids by the lobster. A mixture of two parts cod liver oil to one part corn oil seems to provide an acceptable balance of saturated and unsaturated fatty acids. An optimum concentration of this mixture is around 4 per cent of the dry diet weight (Conklin, unpublished data).

Carbohydrate Requirements

The need for carbohydrates in the diet of crustaceans has not been generally demonstrated. Castell and Budson (1974, J. Fish. Res. Bd. Can. 31:1363-1370) suggested that Homarus americanus did not have the ability to utilize carbohydrate for energy. However, work in our laboratory shows that increasing the carbohydrate levels (starch) improves growth (Conklin, et al. submitted for publication). Presumably, this increased starch content spares some protein which was being used for energy rather than growth.

Vitamins and Minerals

Specific vitamin and mineral requirements have not yet been demonstrated for the lobster. As a general rule, "shotgun" mixtures of both vitamins and minerals are used to supplement formulated crustacean rations. These supplemental mixtures are, in addition to vitamins and

minerals, present in other components of the diet such as yeast, rice bran, shrimp meal, etc. Until purified test diets are developed which eliminate these nondefined components, it will be impossible to define many of these requirements as they are only required in very small amounts. Recently, we were able to show a reduction in mortalities with the addition of vitamins and mineral mixtures used as a replacement for rice bran in the basic E-1 diet (Conklin et al. submitted for publication).

A general problem noted with the use of artificial diets for juvenile lobsters has been a high incidence of animals with a soft shell. Lobsters with this soft or improperly formed exoskeleton appear to be highly susceptible to infections of chitinolytic bacteria which are normally nonpathogenic (op. cit.). Fisher and coworkers (25) proposed that the lack of some nutrient in the artificial diets interferes with epicuticle repair mechanisms. Preliminary work by Castell and Covey (1976, J. Nutr. 106:1159-1165) suggests that a vitamin D deficiency may upset calcium metabolism and the normal development of the exoskeleton. Gallagher, in studying calcium/phosphorus (Ca/P) ratios of artificial diets, found that a Ca/P ratio of 1.55 or above led to abnormalities in the juvenile lobsters endocuticle. Viewing the above-mentioned works as a whole, it seems clear that this abnormal soft shell condition is related both to mineral and vitamin D deficiencies or imbalances. Experiments to pinpoint the optimum balance of these factors will be carried out in the near future.

Work in progress indicates that diets incorporating all of the factors discussed produce growth rates which are equal to that produced

by live brine shrimp. It is hoped that the further experiments planned around the vitamin and mineral requirements will eliminate the abnormal shell problem. If this proves to be correct, we will have available, for the first time, a suitable artificial diet which can be used to carry out the needed research for commercial diet development.

Future Work

The final steps leading to commercial diet development have been defined in a review on lobster nutrition (26). Definitive research will be needed in three major areas before routine formulation of least-cost diets from commonly available feed ingredients can occur. These three areas of investigation are as follows:

1. Definition of changes in quantitative nutrient requirements throughout the life cycle of the lobster. General nutrition studies indicate optimum nutrient levels during an animal's life span continually change. While work with juvenile lobsters (up to four months of age) is probably suitable in defining qualitative nutrient requirements, further work will be necessary to determine optimum quantitative requirements for larger animals.

2. Definition of optimal quantities of both essential and growth promoting nutrients. In order to maximize the rate of growth, it will be useful to examine nutrient interaction. If the experiences of farm animal nutrition can be used as a guide, this effort will be a continuous process.

3. Translation of results derived with purified constituents into a form useful to the commercial feed industry. Coefficients of digest-

ibility, usable nutrients, and energy content of feedstuffs in relationship to the lobster will have to be compiled just as they have been for farm animals. The necessary flexibility of least-cost diet formulation is dependent on being able to match nutritional requirements of the animal with the appropriate percentages of these feedstuffs that are available for use.

GENETICS

The future of lobster aquaculture is usually envisioned as a highly mechanized, intensive technology analagous to today's chicken industry (26). Because of the relative ease of rearing larvae, the acceleration of growth at increased temperatures, the relative hardiness of the animal in the laboratory, and the high economic value, the American lobster is an ideal candidate species for a multidisciplinary research project aimed at the development of such intensive aquaculture technology. Yet, this vision must be tempered with the knowledge that Homarus is both an endangered natural resource and a species still far from domestication. We depend on the productivity and genetic potential of heavily fished natural populations. Thus, our studies encompass the genetics of natural as well as laboratory populations of lobsters. Our results are applicable both to intensive culture and to many problems encountered in extensive aquaculture schemes such as ocean ranching of salmon (Joyner, 1975, Jour. Fish. Res. Bd. Canada, 33:902-904) and pond and ocean stocking of shrimp (Shigueno, 1975, Shrimp Culture in Japan).

The state of the lobster fishery has, of course, been well studied and documented. Suffice it to recall two points relevant to the development of the genetics program area at the Bodega Marine Laboratory. First, fishing pressure has produced a demographic shift towards populations composed of younger, smaller lobsters (Skud, 1969, Fiskeridir, Skr. Ser. Havunders 15:295-309). These produce fewer eggs, and with reproductive output thus impaired, lobster populations seem to be on the verge of precipitous declines. The establishment of lobster breeding colonies may

ultimately be as important to the preservation of genetic resources as it is to the growth of aquaculture per se. To date, complete control over lobster reproduction has not been achieved, and the problem of broodstock development is currently the primary focus of this program area. We shall return to a discussion of this problem and the evolution of our research program later.

A second aspect of the lobster fishery and natural resource that initially concerned us was the rather bitter controversy between the traditional inshore, pot fishery and the newer offshore, trawling fishery. The latter had blossomed with the discovery, in the 1950s, of virgin populations of large lobsters in the deep canyons off the continental slope. When studies showed that adult offshore lobsters migrate up onto the continental shelf in spring and summer when extrusion of eggs and mating occur, controversy centered on the role of offshore stocks in the reproduction of inshore populations. However, this was not only a question of importance to the management of the fishery. Clearly, if the American lobster was subdivided into genetically different stocks, it would be important to examine the aquaculture potential of these separate stocks. Already Rodgers, Cobb, and Marshall (1968, Proc. Natl. Shellfish Assoc.) had demonstrated differences in the sizes of inshore and offshore lobsters. Could these reflect different growth rate potentials?

This was the historical, resource context in which the aquaculture project at the Bodega Marine Laboratory and, in particular, its genetics program area developed. In the next section we shall discuss the general problem of genetics research in aquaculture, the specific

questions about lobsters we initially attempted to answer, and the methodological approaches taken. Results of our studies of biochemical genetics in lobsters and their application to aquaculture are summarized in later sections. In a fifth section, we go on to some more traditional quantitative genetics of growth rate and discuss the implications for selective breeding. Finally, we conclude with a review of the need and current status of lobster broodstock development and a look into the future of research in this area.

What is the Role Of Genetics Research In Aquaculture?

An immediate response to this question often is, "To breed bigger, tastier, faster growing, disease resistant, and economically more efficient stocks." Of course, such great expectations are understandable in light of the tremendous impact that genetics research has had on crop and animal improvements. And there is no question that a rational genetics breeding program is an ultimate desideratum for any aquacultured species. Nevertheless, because many of the prime candidates for aquaculture (particularly invertebrates such as the American lobster) are not yet domesticated, traditional approaches and methods of animal breeding cannot be applied. So, must aquaculture genetics research await control over reproduction? Certainly not. Basic problems can be addressed directly.

Genetic improvement can only take place if there are genetic differences among individuals. Therefore, the primary focus of any aquacultural genetics research program must be the description and quantification of genetic variation. At the start of our program, little was known about genetic variation in lobsters, and three fundamental questions

concerning variation required answers: 1) How much genetic variation exists among individual lobsters? 2) Are there different stocks of lobsters and are inshore and offshore lobsters the same stock? 3) How different are the European and American lobsters and might hybridization of these two species be beneficial? Within the last 10 years, techniques have emerged to provide rapid, preliminary answers to these types of questions. These techniques are chiefly biochemical - the separation by means of gel electrophoresis of protein or enzymes extracted from the homogenized tissues of organisms and the visualization of specific enzymes by chemical assays. Our first task then was to apply these techniques to the American lobster and to measure variation.

Biochemical Genetics Of Homarus Lobsters

The theoretical basis for using electrophoresis to detect genetic variability is the now well-confirmed relationship between the genetic material, DNA, and the specification and synthesis of cellular proteins. A chief advantage of the technique is that the characters or phenotypes studied, distances that enzymes move in an electric field (electrophoretic mobilities), are under simple and direct genetic control.

Phosphoglucomutase: A case study

The inheritance of enzyme phenotypes in the lobster Homarus are typified by our studies of phosphoglucomutase (PGM), a major enzyme of carbohydrate metabolism. Gels that have been electrophoresed and stained for PGM activity show two distinct zones of activity that are under separate genetic control. All American lobsters studied thus far have the same band of activity in the faster-migrating PGM-2 zone, and we assume that there is a single, nonvariable gene locus directing

the synthesis of this enzyme. (Note that traditional genetics methods treat only phenotypic differences such as eye colors, seed shapes, etc., but that biochemical genetics techniques also detect and quantify phenotypic and genetic similarities such as PGM-2.) In the slower-migrating zone, PGM-1, three different band patterns or phenotypes are found. Two are single band phenotypes that differ in mobility by 3 mm; the slow band phenotype appears in about 54 per cent of all lobsters and is arbitrarily called PGM-1^{100}, while the fast band phenotype, called PGM-1^{103}, is found in only 7 per cent of lobsters. The remaining 39 per cent of lobsters appear double-banded, having both PGM-1^{100} and PGM-1^{103}. Our genetic interpretation of these phenotypes is as follows: the two kinds of single-banded individuals are homozygotes, i.e. carrying either two 100 or two 103 genes (alleles) at the Pgm-1 locus, while the double banded individuals are heterozygotes carrying one 100 and one 103. This hypothesis of classical Mendelian inheritance has been fully confirmed by analyses of females and their offspring (13,24). A single case of a non-Mendelian PGM-1 phenotypic ratio in a lobster family has been ascribed to multiple insemination (Nelson and Hedgecock, American Naturalist, in press). Thus, our studies of PGM have enabled us to 1) identify a specific gene in the lobster, 2) type individuals by their PGM phenotype, and 3) demonstrate that more than one male may successfully fertilize a female's eggs.

PGM-1 is only one of 46 gene-enzyme systems (loci) we have studied in Homarus (Table 1). We have confirmed by progeny analyses Mendelian inheritance for a total of nine enzymes; these are the first gene loci to be formally described in the lobster Homarus (13,24). In a later

Table 1. Gene-enzyme variation in Homarus lobsters. An enzyme is considered variable if more than one electrophoretic form has been detected. Final column indicates those enzymes having completely different electrophoretic profiles in the European and American species.

Enzyme	*H. americanus* Number of Gene Loci	*H. americanus* Variable ? (fraction of loci)	*H. gammarus* Number of Gene Loci	*H. gammarus* Variable ? (fraction of loci)	Species Different?
Acid phosphatase	5	Yes (1/5)	5	Yes (3/5)	Yes (2/5)
Esterase	7	Yes (5/7)(2/7*)	5	Yes (1/5)	No
Fumarase	2	Yes (1/2)	2	No	No
Glutamate-oxaloacetate transaminase	1	Yes	1	No	No
Glyceraldehyde-3-phosphate dehydrogenase	1	No	1	No	No
Hexokinase	3	Yes (1/3*)	3	No	No
Isocitrate dehydrogenase	1	Yes*	1	No	No
Leucine aminopeptidase	1	No	--	--	--
Malate dehydrogenase	2	Yes (2/2)(1/2*)	2	No	No
Malic enzyme	1	Yes	1	Yes	No
Mannose-6-phosphate isomerase	1	No	1	No	No
Peroxidase	1	No	1	No	
6-Phosphogluconate dehydrogenase	1	No	1	No	No
Phosphoglucose isomerase	3	Yes (2/3*)	3	Yes (2/3*)	Yes (1/3)
Phosphoglucomutase	2	Yes (1/2*)	2	Yes (2/2)(1/2*)	No
Tetrazolium oxidase	5	No	5	No	No
Triosephosphate isomerase	1	Yes	1	Yes*	No
Tetrazolium reductase	2	Yes (1/2)	1	No	No
	40		36		
Protein (Coomassie stain)	6	Yes (4/6)	6	No	No

*Fraction of variable loci whose inheritance has been confirmed by progeny analyses.

section we will discuss how these genes may be useful in aquaculture broodstock development.

Genic Variation Within Lobster Populations

Initial answers to the three fundamental questions stated above were obtained partially in a study of 290 mature H. americanus drawn from eight localities on the Atlantic Coast (16). In a subsequent study, specimens of H. gammarus were obtained from the Irish Sea and the waters of Norway for comparison to American lobsters (38).

The amount of genetic variation among the individuals of a population may be quantified in a number of ways. First, what proportion of gene-enzyme systems show variation? If one considers all variation, this measure is highly dependent on sample size as reflected in the data of Table 1. Here the American lobster appears to have more variation than its European cousin, but actually much of this variation is due to rare alleles detected in the larger samples of H. americanus. This bias is somewhat remedied by requiring that for a gene-enzyme system to be called variable or polymorphic, the most common allele may have a frequency no higher than, say, 95 per cent (see Table 2).

Second, what is the average number of alleles detected per gene-enzyme system? Again, this measure depends on the number of individuals studied.

Finally, what is the average proportion of gene loci at which an individual carries two different allelic enzymes, i.e., the proportion of heterozygous loci per individual? This may be estimated from the proportions of heterozygotes observed at each locus. For the two PGM

Table 2. Summary statistics of genetic variation in average populations of the American and European species of Homarus. Data from 8 populations of H. americanus (16) and 2 populations of H. gammarus (38).

Statistic	H. americanus	H. gammarus
Number of loci studied	44	41
Average number of individuals sampled per locus	30	24
Proportion (%) of polymorphic loci*	14.1%±1.5	13.1%±2.3%
Average number of alleles per locus	1.23±0.03	1.21±0.08
Average proportion (5) of heterozygous loci per individual	4.4%±1.1%	3.9%±0.5

*A locus is defined as polymorphic if the frequency of the most common allele is no greater than 95 per cent.

examples given, an individual has a probability of 0.39 of being heterozygous at Pgm-1 but zero probability of being heterozygous at Pgm-2. These probabilities are averaged over all loci studied to obtain a measure of variability that is more dependent on the number of gene-enzyme systems studied than on the number of individuals.

By all three statistics, populations of the American and European lobsters have similar amounts of genetic variability (Table 2). Average heterozygosity in lobsters is approximately the same as in mammals, and thus, there appears to be enough variability for artificial selection to be effective. As we shall see, this has been substantiated in an entirely different way by a study of growth-rate variation. On the other hand, our results showing about 4 per cent heterozygous loci per lobster were, at first, startling. Most invertebrates that had been studied up to that time were found to have large stores of variation, with average heterozygosity in the neighborhood of 15 per cent. In order to confirm our observation of low variation in lobsters, we conducted a survey of variation in other decapod crustaceans, particularly species having economic importance such as Cancer magister. We have found low variation to be the rule in the decapods.

The adaptive and biological significance of genic variation as measured by electrophoresis is currently a subject of much interest to specialists in the fields of population and evolutionary genetics. Data generated by our sideline investigation of decapods have played a role in the development of this field (cited in the review of Selander, 1976; also by Valentine, 1976; both in Molecular Evolution, F.J. Ayala, Ed.). We have pointed out elsewhere the relevance that a unifying theory of

genic variation might have for selection of aquaculture candidate species (24; Nelson, K., Proc. of the 8th Annual World Mariculture Society Meeting, submitted for publication).

Are there different stocks of lobsters?

For each gene-enzyme system studied in the eight population samples of H. americanus (Fig. 4), we asked whether the frequencies of alleles differed from locality to locality. In almost all cases, they did not. The frequency of the Pgm-1^{103} allele, for example, varied from a low of 17 per cent in the Maine sample (MIA, Fig. 4) to a high of 31 per cent in the Bay of Fundy sample (BFY), a nonsignificant difference. Frequencies of malic enzyme variants, however, did vary significantly. A fast allele, Me^{102}, had a frequency of 97 per cent in the Gulf of St. Lawrence sample (GSL), but a frequency of only 2 per cent in the Massachusetts inshore samples (WHP, MVS). In our offshore samples (GBS, LSA) the Me^{102} allele had a frequency of 45 per cent. Because such genetic differences could only be maintained by unrealistically high rates of natural selection if genes are truly exchanged among populations, the Me results cause us to reject the hypothesis that offshore lobsters repopulate the inshore areas. Different stocks of lobsters do exist, and this must be taken into account in lobster aquaculture.

Another statistic, I, summarizes allele frequency information and measures the overall degree of genetic similarity between populations on a scale from 0.0 to 1.0. When calculated for the Pgm-1 comparison between MIA and BFY, it yields a value of 0.98. In the cases of the Me comparisons, GSL vs MVS and MVS vs LSA, I is 0.06 and 0.79, respectively. Average genetic similarity for all loci and between all populations

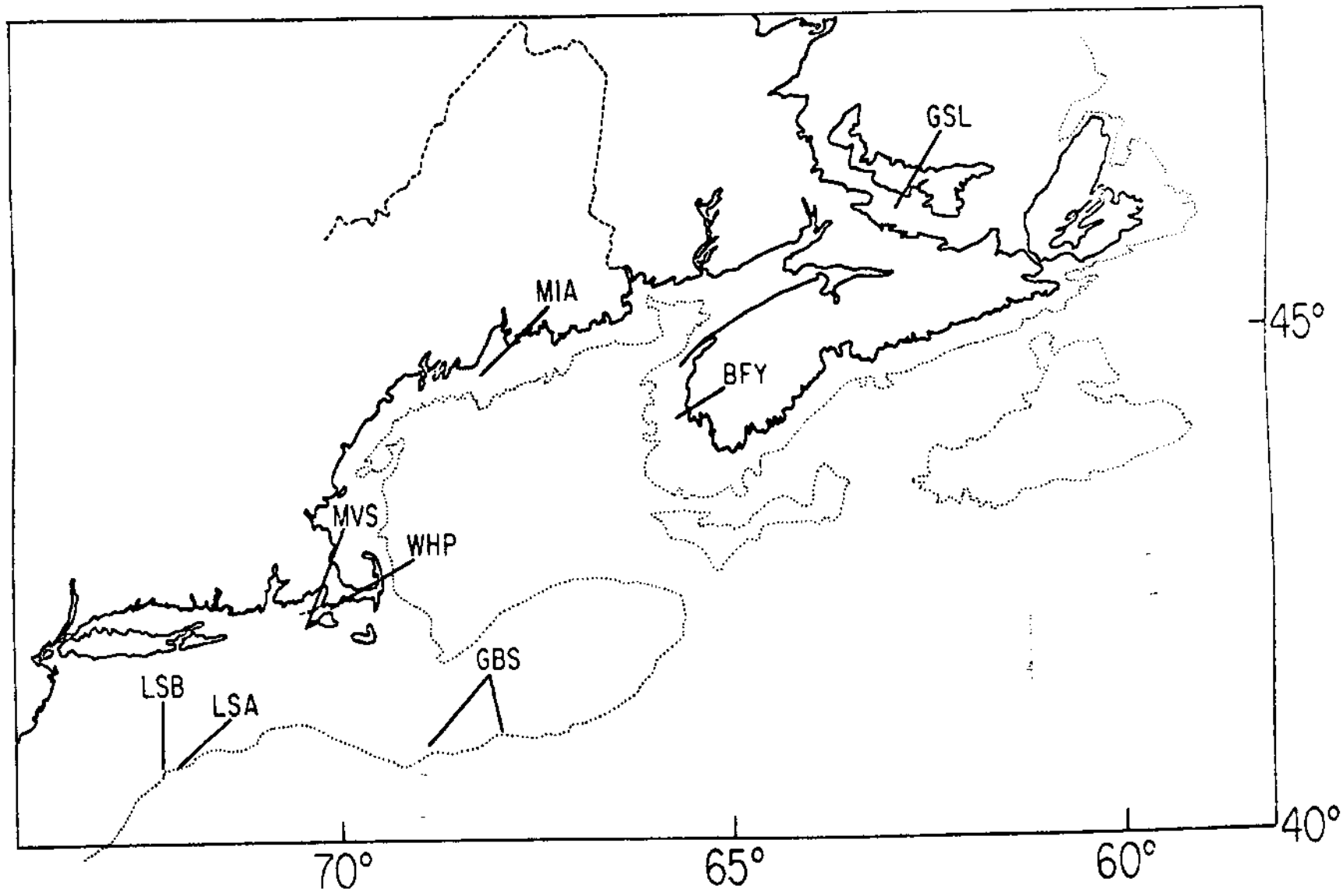

Figure 4. Sampling localities for Homarus americanus. From north to south they are: Gulf of St. Lawrence (GSL), Bay of Fundy (BFY), Gulf of Maine (MIA), Woods Hole, Mass. (WHP), Martha's Vineyard, Mass. (MVS), offshore Georges Bank (GBS) and Hudson Canyon samples (LSA and LSB).

studied in the American lobster is $\underline{I}$ = 0.994±0.001. Thus, although gene exchange among populations from different geographical regions appears to be limited in some way, these stocks still have very similar gene pools.

In a subsequent study of the European species (38) comparable results were obtained. Average genetic similarity between the Norway and Irish Sea samples was 0.987. Again, the greatest divergence of allele frequencies occurred at the Me locus, and again, Me^{102} occurred in much higher frequencies in the Norway sample. This suggests population subdivision on a large geographic scale and a possible selective advantage for the Me^{102} allele at higher latitudes.

Genetic similarity of European and American lobsters

Electrophoretic techniques allow one to quantify the number of gene differences between species in just the same way as in the comparisons between stocks of the American lobster. Our results indicate surprisingly great similarity between H. americanus and H. gammarus, with $\underline{I}$ = 0.896±0.007. Only three gene-enzyme systems have evolved completely different electrophoretic profiles (Table 1). Based on evidence from other animals, we have speculated that this similarity is the result of rather recent evolution of these lobsters. Moreover, within the past two years, matings between these species have resulted in successful extrusions both in our laboratory and in the lobster facility at San Diego State University. The latter group has also produced viable, healthy hybrid lobsterlings. Thus, the American and European lobsters are similar enough to crossbreed but different

enough at the gene level so that hybrid progeny may be expected to show hybrid vigor with respect to aquaculturally important traits. We shall return to the use of interspecific hybridization in a later section.

Gene-enzyme Markers and Their Applications in Aquaculture

The basic BML Sea Grant mission over the past five years has been the development of the science and technology of aquaculture using the lobster Homarus as a model. Traditional methods for broodstock development were precluded by the absence of husbandry techniques, and the literature on the genetics of Homarus consisted of one report of chromosome numbers and some work on esterase variation. Lobster, indeed, presented a challenging model from the standpoint of genetics. As indicated, our approach was, first, to recognize and to formulate fundamental questions concerning genetic variation in natural populations, and second, to answer those questions rapidly with the available methodology of biochemical population genetics. To lay aquaculturists and even to scientists unfamiliar with recent advances in this specialized field of genetics, the relevance of these studies to aquaculture might not have been immediately apparent. However, not only did this methodology allow us to answer our initial questions and increase our knowledge of stock and hybridization potential, but it also opened up a completely new technology with varied and far-reaching, powerful applications for all crustacean aquaculture (24; Hedgecock, Proc. of the 8th Annual World Mariculture Society Meeting, submitted for publication).

These applications may be placed in the context of four problems that are encountered in the development of aquaculture broodstock:

1) taxonomic identification of the cultivated species, subspecies, race

or ecotype, and organisms associated with these such as parasites, 2) family or cohort identification such as required in hatchery release and growout programs, 3) accuracy in pedigree data, and 4) monitoring of inbreeding and choice of optimal lines for crossbreeding (op. cit.).

The properties of gene-enzyme variation and the ease with which it is detected by gel-electrophoretic methods offer technological solutions to these problems. Enzyme variants can serve as markers of individuals, populations, stocks, or species because of their detectability in milligram biopsy samples, their stability over a broad range of developmental and physiological states and their adherence to the laws of Mendelian and population genetics.

Specific examples of each of these applications have been published and will not be elaborated here. It must be emphasized, however, that for crustaceans, biochemical genetic tagging appears to be the only practical means for identifying hatchery reared stocks in the ocean, and thus, the only means of evaluating survival, dispersal, and recapture rates in lobster, crab, or shrimp hatchery-restocking programs.

Genetics and Lobster Growth

Description and quantification of genetic variation in lobsters were also extended to the more complex but economically important phenotype of growth rate. In this case, genetic variation cannot be measured directly as with enzyme variation. Therefore, we attempted to estimate variation in growth rates of lobsters and the proportion of this variation attributable to additive genetic variation. This proportion, known as the heritability, tells us the extent to which selective breeding might improve the growth rate of lobsters. Such information,

together with our expectations for hybridization, is essential in formulating future research and breeding programs.

The first experiment was simple in design (28), but yielded a preliminary estimate of growth-rate heritability and several new questions for future research. An interesting complication in the experimental design was that hatching took place over a period of 45 days, so progenies entered the experiment at different times. Average family growth rate declined in an inverse-rank correlation with time of introduction to the seawater tables. Deteriorating water quality coupled with increasing lobster biomass and bacterial levels over the course of the experiment are thought to have been the cause of poorer growth. Similar environmental factors were also thought responsible for growth-rate differences between siblings reared in two different seawater tables.

Further analyses to detect differences among families were confined to groups of families placed in the experiment within the same week. There was considerable variation in the growth rate of animals treated alike - the standard deviation of juvenile average weight increase per day was over 30 per cent. In turn, up to 30 per cent of this growth rate variation seemedto be heritable. Though preliminary, the estimated heritability of lobster growth-rate is comparable to those found in domesticated animals such as cows, pigs, and even rainbow trout. This result supports the suggestion from our biochemical studies that lobsters, once domesticated, have the genetic potential for significant improvement through selective breeding.

This pilot study spawned two follow-up experiments. One was designed to test competing hypotheses for the observed negative correlation between

the growth of a lobster and total biomass of lobsters in nearest neighboring compartments (Fig. 5). Since lobsters were grown in clear plexiglass compartments, the effect could have been a behavioral response mediated by visual contact among neighboring lobsters. Alternatively larger lobsters might have inhibited the growth of smaller neighbors through increased metabolite concentrations. The experimental design was to grow sibling lobsters with zero, three, and five close neighbors, both in clear and in opaque compartments. Unfortunately, the results were inconclusive. The number of neighbors had no effect on growth rate and lobsters in clear compartments grew slightly faster than those in opaque cells. This latter result was quite unexpected, but may have been due to slightly smaller dimensions in compartments constructed of thicker, opaque plastic material. The effects of metabolite loads or behavioral interactions on lobster growth thus remain open questions.

Another experiment that was designed and initiated in July 1976, as a result of a pilot study of growth-rate variation, involved over 3,000 lobsters from eight families grown at three different temperatures and on four different diets. Executed in collaboration with the nutrition group of our project, this experiment was made to evaluate the nature and extent of interactions among genetic, temperature and diet factors. Unpredictable family x environment interactions had been observed in the pilot study (28). If widespread, such interactions could severely limit the validity and generality of studies concerning the effects of temperature or diet on growth. The experiment was recently concluded, and the data have not yet been fully analyzed.

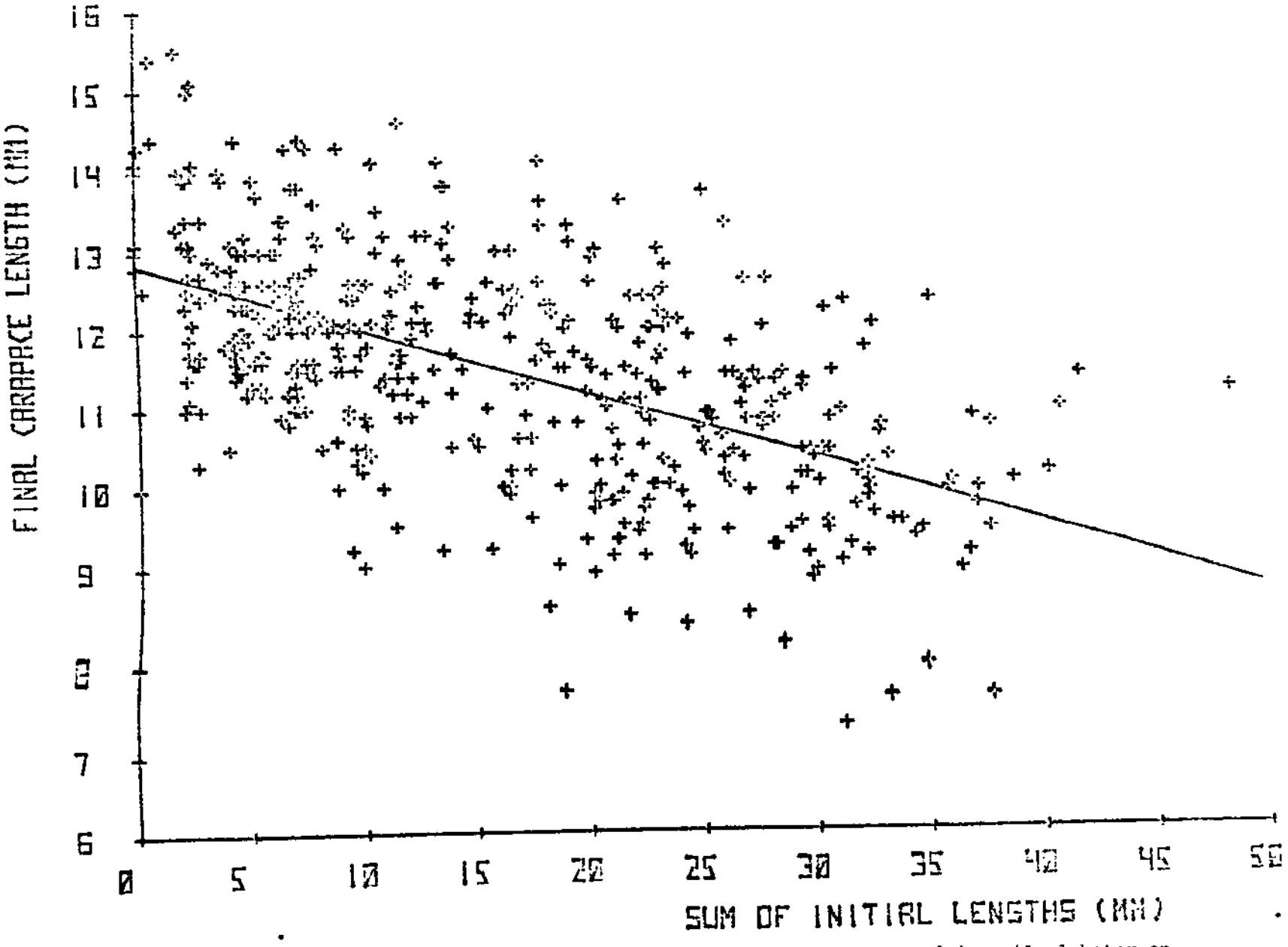

Figure 5. Regression of the final carapace length of each experimentally reared juvenile lobster on the sum of the carapace lengths of its nearest neighbors at the time it was placed in the experiment.

Lobster Reproduction and Broodstock Development

The development of lobster aquaculture depends upon controlled production of young from broodstock. There are three ways to acquire ovigerous females: 1) obtain the egg-bearing lobsters from fishermen specially licensed to trap berried females, 2) impound mature, non-ovigerous females in late summer and early fall and 60 to 80 per cent of these will extrude, and 3) contain the whole mating-extrusion-hatching cycle in an artificial environment (21). The first source is highly seasonal and unlikely to be available to commercial aquaculturists. The second source is legal, but, again, highly seasonal. Some control over extrusion and hatching may be possible by temperature manipulation but Dr. D. Aiken has pointed out the dangers of interference from molt cycle disturbances. The third alternative, complete control over reproduction, requires an investment in research and broodstock maintenance but will likely give a higher return in terms of steady production of larvae and the possibility for selective breeding. Consequently, in mid-1975, we began to turn our attention to the problem of lobster reproduction. The following is a brief summary of relevant biology and our experimental approach to the problem of reproductive control.

In the natural environment, nearly six years is required to reach the size of sexual maturity. Following a female's first "nuptial" molt (when insemination occurs), nine months typically elapse before extrusion (fertilization) of eggs (Hughes, 1973, S-E-A Scope 3:6-8). Most of the larval history of Homarus is then passed within the egg in contrast to other crustaceans which endure a long planktonic larval life. At ambient temperatures (Massachusetts) or at a constant 10°C the female retains

the eggs for nine months before hatching. Thus, under the ambient temperature regime of New England, 18 months pass between fertilization and hatching. Under certain conditions, this time may be reduced even in the natural environment. The time between extrusion and hatching, for example, may be reduced to only four months at 20°C (Hughes, 1973, S·E·A Scope 3:6-8; Perkins, 1972, Fisheries Bulletin 70:95-97). However, control over extrusion will offer more flexibility in egg production.

Ordinarily, in New England waters, the nuptial molt and insemination occur in the summer or early fall, but extrusion and fertilization of eggs is put off until the following spring or summer. However, as many as 10 or 20 per cent of these females extrude during the (same?) fall or winter (Herrick, 1911, Natural History of the American Lobster), and it is at least likely that ovarian development in these fall-extruders has been accelerated to meet the next spring's hatch (Perkins, 1972). According to Aiken (personal communication), a large proportion of the one and one quarter pound hens (2nd nuptial molt?) from the waters of Prince Edward Island, when brought into the laboratory during the summer, extrude in the early fall. Here at the Bodega Marine Laboratory, five matings resulted in extrusion within five months, while one mating resulted in an extrusion after one year. The impression from the literature is that whereas the extrusion-to-hatch time is more or less strictly (inversely) related to temperature, the time from nuptial molt to extrusion is more "dichotomously variable," with extrusion and fertilization time more probably a close function of the energetics of ovarian development and only indirectly related to time of nuptial molt and insemination. If ovulated eggs are available in fall, extrusion may occur then; otherwise, it is postponed

until the following summer. Thus, we have concentrated our experimental efforts on the events leading to extrusion.

The complex interplay of ovarian maturation, the molt cycle, and extrusion itself appears in various Crustacea to be under the influence of photoperiod and temperature; other factors such as diet and water depth play a role. Hughes (1973, S·E·A Scope 3:6-8) states that a 46 cm water depth is essential for extrusion to occur. A broodstock facility was, therefore, built consisting in the main, of 72 individual holding tanks 37 cm by 74 cm by 49 cm deep as described above; this last figure reflects Hughes' observation.

For experiments, these holding tanks are connected in eight batteries of nine tanks each. Any battery may be held on either an ambient, flow-through or a heated, recirculating regime. These eight batteries, in addition, may be held on as many as six different photoperiods. In our first experiment, batteries were switched from ambient, flow-through ($14^{o}C$) to recirculating, heated water at two different times, spaced 45 days apart. By increments, the animals were acclimatized at $20^{o}C$ in the re-circulating systems. Two different photoperiod regimes were combined with each temperature regime to provide a total of four experimental conditions, each covering nine animals. The experimental subjects consisted of 36 females mated in our laboratory within the preceding six months, all balanced over the four experimental conditions according to time and genetic nature of the mating. Animals were visually checked for extrusion and fed daily on an "optimal" diet of bottom fish, squid, shrimp, and shellfish as available.

In this initial study, a succession of system problems stressed the animals severely and only one H. americanus female (mated to an H. gammarus male) extruded in September 1976. This large complement of hybrid eggs, the first to be extruded at the Bodega Marine Laboratory, is due to hatch within several months, and the growth of the juveniles will be compared with H. americanus progenies hatching at the same time.

Currently, this facility is being replumbed and safety features are being installed to ensure that system difficulties are kept to a minimum. The experiment has been expanded to include 72 wild-caught, recently mated females, balanced over four regimes of temperatures and two of light. This experiment will continue through summer 1977. Vitellogenesis is being monitored by the serum titer of female specific protein.

Since August 31, we have had six extrusions in addition to the hybrid cross reported above. Of these, one was an American x American cross, one was an American female x European male and four were European females x American males. Lobster workers at San Diego State University have also had several extrusions by European females mated to American males. We are encouraged by these initial successes in hybrid crosses, particularly those in the direction European female x American male. Hybrid crosses appear to offer, in the near future, a source of genetically defined juvenile stocks for continued research in the nutrition and disease areas.

SYSTEMS ANALYSES AND ECONOMICS

Introduction - Early Goals and Objectives

In the initial phase of the project, as large scale commercial lobster aquaculture seemed to be biologically possible, the question of how much it would cost became increasingly important. There were no good estimates of culture cost, but there were many suggested "best" methods of culture or ways of improving culture methods through research. However, these were often evaluated in the limited context of the researchers' areas of expertise. Because of the wide variety of backgrounds among those involved, there were many different opinions as to the best method of culture and highest research priorities.

An obvious need existed for a means of evaluating the state of knowledge of lobster aquaculture and how it might best be improved. Because the final goal was to develop the technology for commercially viable aquaculture, it seemed most reasonable to evaluate these issues in an economic context, i.e., in terms of the production cost of lobster aquaculture. The tool used in these evaluations could not merely sum the various costs of one culture scheme. It had to be general enough to reflect a variety of culture methods and flexible enough to incorporate advances in research as well as demonstrate their effect on culture methods and cost.

The approach taken was to develop a general mathematical model of a lobster culture facility. This model included descriptions of the lobsters response to the system environment, the physical system necessary to provide this environment, and the cost associated with each

component of the system. The model of the biological responses of the lobster was designed to be general enough to apply to any conceivable culture environment. The physical system was modeled so that with a minimum of changes, it would reflect any one of a variety of culture schemes to be considered. The costs of various components were modeled so that they depended on the operating level of that component (e.g. the cost of pumping depending on the product of flow rate and lift).

The development of the model had a threefold purpose: 1) to project culture costs and determine the accuracy of projections, 2) to determine optimal (least-cost) culture methods, and 3) to determine the best path of research through sensitivity analysis of culture cost to poorly known or economically important parameters. The general philosophy of model development was to construct a preliminary model based on assumptions where experimental data were lacking, to exercise it to determine the characteristics of the system, and to improve it in the areas of greatest economic importance. Through this process, an increasingly valuable tool of analysis has evolved.

Development of the Model and Techniques of Analysis

The initial effort in describing the response of the lobster to its environment was to model the dependence of growth rate on water temperature. Molt data from several hundred lobsters grown at the Massachusetts State Lobster Hatchery on Martha's Vineyard were analyzed. In order to formulate this model, the process of growth by successive molting had to be described. A model of growth was developed which translated this basically discrete process into an economically meaningful, continuous process of weight increase. Since growth is measured experimentally in

terms of carapace length, a method of converting these measurements to weight was derived. The outcome was a differential equation describing growth rate in terms of weight as a function of weight and temperature.

After growth rate had been related to temperature the optimal culture temperature could be determined. This process is a good example of the benefit of applying optimization theory to aquaculture. Lobsters, like most poikilotherms, grow faster at higher temperatures. Yet, heating water causes an increase in operating and capital costs. There is, therefore, a trade off between higher cost per unit time for a short period and lower cost per unit time for a long period. The temperature which will produce a lobster for the least cost will, in general, vary with time over the culture period.

A preliminary model of culture costs which included the cost of heating water, weight-dependent costs, and costs which depended only on time was formulated. With these models the optimal temperature (i.e., the temperature which minimizes total culture cost) was determined using optimal control theory (2). This is a technique of mathematical optimization which determines an optimal function of time rather than a single value (i.e., the optimal temperature at each point in time during the culture period was determined). Dr. Rauch of Lockheed Research Laboratory in Palo Alto provided assistance in this work. The general result was that the optimal temperature was either the temperature of maximum growth rate or the natural seawater temperature, depending on the relative costs of heating water. In cases where the costs were such that the optimal temperature changed during the culture period, it always changed from the maximum growth temperature down to the natural temperature.

This indicates that it is best to provide conditions of high growth rate while lobsters are small and possibly relax these conditions as they become larger (2).

The next major effort was to extend the model to include more biological responses, environmental variables, detailed physical system, and specific dependence of cost on system operation. In addition to growth rate, the biological part of the model included the rates of mortality, ammonia and solid waste excretion, and oxygen and food consumption. The environmental variables affecting these rates were temperature, container size, amount of food fed, and the type of food. While some of the relationships were based on assumptions rather than experimental data, the model and ensuing optimization were developed as an example of how all relevant factors affected the system.

The physical system (Fig. 6) was modeled as a rearing unit which was capable both of pumping water from the sea and returning it, and recirculating it through a waste treatment system for re-use, so that the optimal amount recirculated could be determined on an economic basis (3). The relationships between flow rate and metabolites were adapted from a mass balance relationship developed by Liao and Mayo (Aquaculture, 1, 1972). Culture costs included costs of pumping, heating, waste treatment, space (containers, tanks, building, land), food, feeding and labor. Equipment costs included estimates of amortized capital, operating and maintenance costs.

Optimal-control theory was used with this model to determine simultaneously the optimal values of temperature, container size, amount of food fed, type of food, and recirculation rate (3,14). Results computed

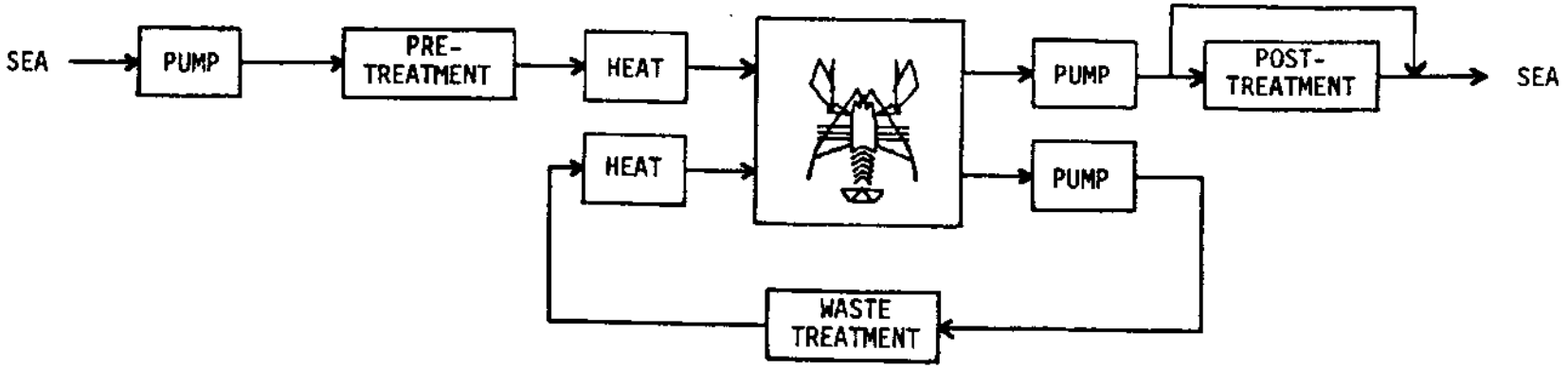

Fig. 6. System diagram.

for exemplary parameter values indicated that in the case of temperature, space, amount of food and type of food, it was best to provide conditions for rapid growth to younger lobsters and relax these conditions later in the culture period. In cases where temperature switched from the maximum growth temperature to natural, the fraction of total flow recirculated switched from one to zero. Dr. Rauch again contributed significantly to formulation of this model and the optimal control analysis.

At this point, experience gained through modeling and computation of costs for optimal culture systems enabled us to recommend areas of future research which had the greatest impact on culture costs. Since natural foods were prohibitively expensive, development of a good artificial food was a necessity. Because flow rates and, therefore, heating, pumping and waste treatment costs, depended on metabolite excretion rates and tolerances, these areas deserved greater attention as promising artificial foods were developed. These recommendations were presented at the Lobster Workshop in Bodega Bay, April 1974.

An experiment designed to determine the effect of container size on growth rate was completed about this time. Fifth-stage animals had been grown in five different-sized containers for a period of 10 months. Many experimental problems were encountered in the early phase of this work which cast some doubt on the results, but the data were analyzed and a preliminary model describing the effect of space limitation on growth rate was developed (5) (Fig. 7).

By this time, enough experimental data had accumulated to begin making reasonably accurate cost projections. For these, the parts of the model which were based on experimental data were included (effects

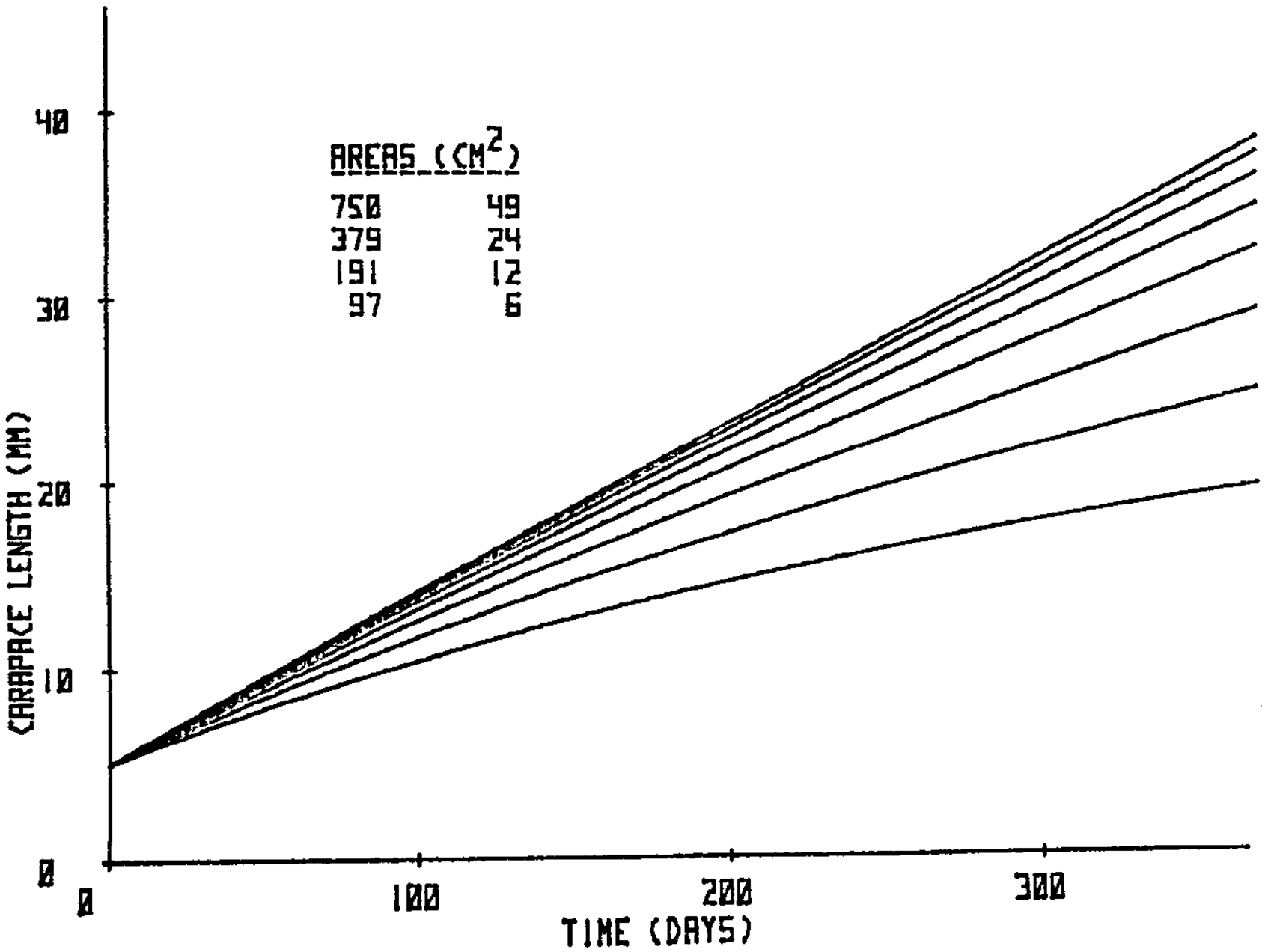

Figure 7. Growth in terms of carapace length for eight different container sizes.

of temperature and space on growth rates, and ammonia tolerance), a good artificial food was assumed, and ammonia excretion rate was based on the maximum available nitrogen. The estimates of costs were improved and extended to include three different types of systems: stacked tray, silo, and raceway. This phase of the modeling was done in conjunction with G. Allen and W. Johnston of the Agricultural Economics Department at UC Davis and was programmed by them for simulation. This is a technique whereby the equations describing a system are solved for specified parameter values (i.e., size of containers, temperature, and recirculation rate are specified for each run as opposed to optimal-control analysis which determines optimal values of these). The ensuing analyses compared the cost of three types of culture systems, showing a stacked tray system to be the least costly. The effects of economics of scale and increased growth rate were also examined. These results were presented at the 1975 Winter Meeting of the American Society of Agricultural Engineers as "An analysis of three facilities for the commercial production of Homarus americanus" by A.M. Schuur, P.G. Allen, and L.W. Botsford.

Following this analysis, optimal-control theory was applied to the three types of systems to determine changes in optimal values of temperature, container size, and recirculation rate (35). Optimal container size was smaller for types of systems with higher relative costs of space. Examination of the effect of varying harvest weight showed that cost per unit weight varied less than 10 per cent over the range of 20 g to 454 g. The optimal number of containers necessary throughout the culture period was determined to be three, by simulating discrete sized containers, rather than allowing container size to vary continuously

(an assumption made earlier to simplify optimal control analysis). The effects of varying some experimentally determined parameters and geographic location were also examined.

An experiment designed to refine our estimate of the effect of temperature on growth rate was completed in our laboratory. Fifth-stage lobsters fed brine shrimp were grown at six different temperatures (15°, 18°, 21°, 24°, 27°, and 30°C) for 120 days. Analysis of results showed highest growth rate at 21°C (average dl/dt = .098 mm carapace length/day) (Fig. 8). The relationship between carapace length and weight was determined for the size range of interest in aquaculture (.06-454 g). These results were combined to develop a model of growth rate in terms of weight as a function of temperature (Botsford, in preparation).

Current Status

The biological model has developed into an accurate useful tool in terms of growth rate, with accurate definition of metabolic rates still partially dependent on development of a good artificial food. The dependence of growth rate on temperature and space accurately describes growth rates observed in experiments in which brine shrimp are used as a food. Comparison of the model with preliminary results from a spatial confinement experiment performed at San Diego State shows close agreement (J. Carlberg and J. Van Olst, personal communications). This not only puts the space model on a firmer basis, but extends the experimental time period on which it is based to one year.

The expression relating oxygen consumption rate to weight and temperature is currently based on experiments by Schuur (unpublished),

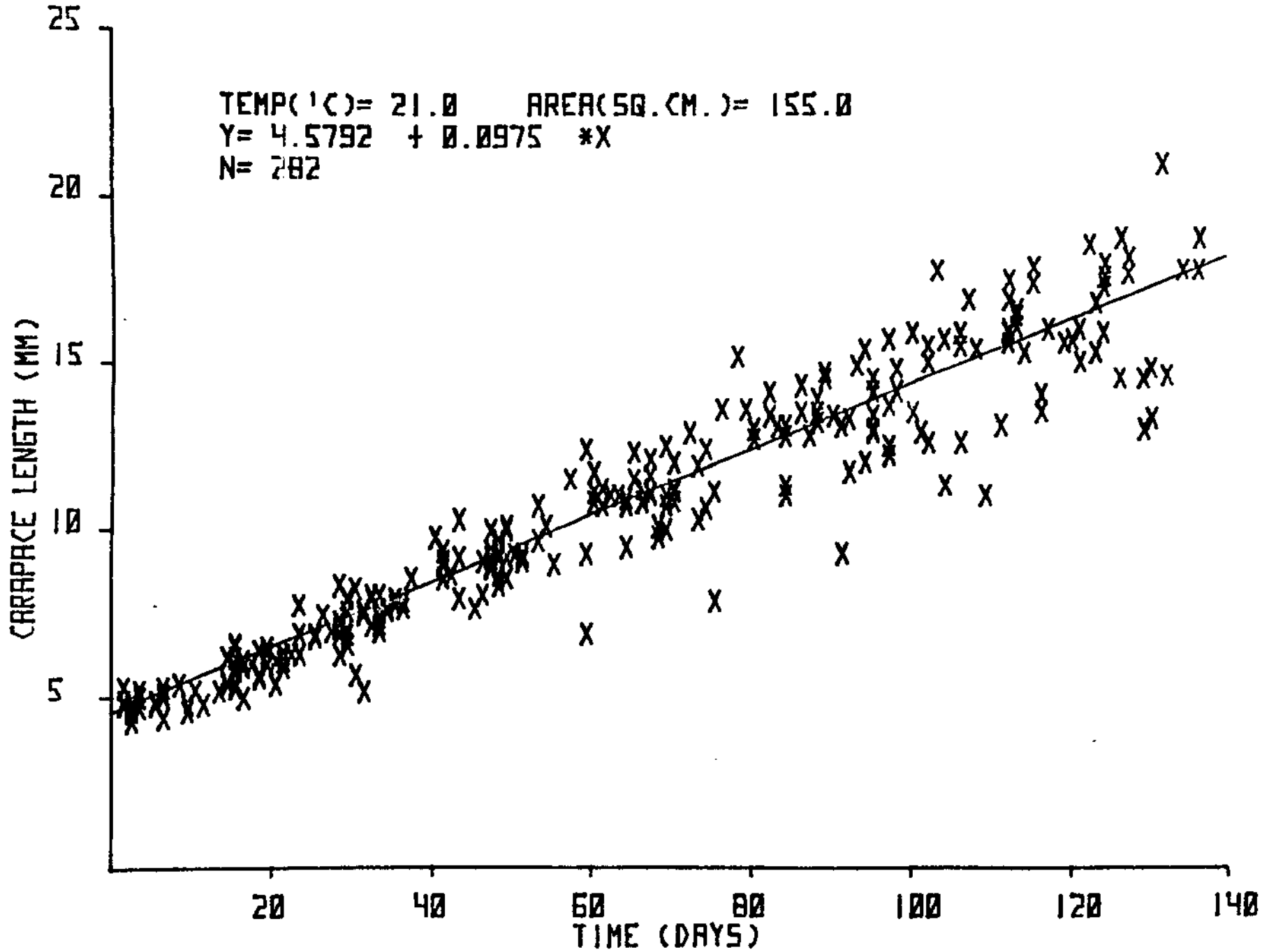

Figure 8. Growth in terms of carapace length at 21°C, linear regression line of slope .0975 mm/day

Logan (1976, Ph.D. thesis, University of Delaware) and McLeese (1964, Helgol. Wiss Meer. 10). Logan and McLeese both found an increase in oxygen consumption rate for fed over starved animals (64 per cent and 75 per cent respectively). The model of oxygen consumption, therefore, is based on McLeese's expression for weight dependence, and Schuur's expression for temperature dependence multiplied by a factor of 1.7 to account for calorigenic energy of feeding.

The food consumption rate expression is currently based on an energy budget and assumed artificial food. Logan (1976) measured the energy requirements of juvenile lobsters in terms of growth, respiration and egestion at 22°C. He found good agreement between predicted and measured consumption rates of brine shrimp. The model of food consumption rate is based on this energy balance. We assume a caloric equivalent for food which is representative of artificial foods. This value varies with specific food type. Changes in the relative amounts of protein, lipids and carbohydrates in the food could alter other terms in the energy balance. Values obtained using this expression for consumption rate are approximately the same as those observed in preliminary experiments and a conversion ratio of 3.6 to 1.

The most important metabolite in terms of the impact on cost is ammonia. The model of excretion rate is a mass balance for nitrogen expressed in terms of the toxic species (NH_3). The model assumes complete deamination of all protein consumed and not devoted to growth. The resulting excretion rate is slightly higher than experimental values obtained by Logan (1976) and in two preliminary experiments performed in our laboratory. More experimental work is needed to define this parameter,

using artificial foods as they are developed. In the base-line model the ammonia concentration is maintained at one-tenth of the 96 hr acute lethal concentration of ammonia for juvenile lobsters (1.2 mg NH_3-N/l) (Gravitz et al., unpublished).

The expression describing mortality is based upon growth experiments at 21°C for animals fed brine shrimp for a period of two to three months. Mortality rate is assumed to be greater than that observed in these experiments for the first three months of growth and decreases in steps as weight increases.

The current model of the physical system is essentially the same as we have described (35). Ammonia is the limiting metabolite which determines flow rate, and oxygen is supplied (as needed) directly to the rearing unit. The outflow is treated to meet EPA standards (9). The model of the physical system is currently being expanded to include alternative methods (to fossil fuel) of heating water such as heat recovery, thermal effluent, and solar heating.

The cost model computes capital, operating, maintenance, and direct labor costs for an indoor, stacked-tray system with a mechanical feeder as described by Schuur et al. (1975, op. cit.). The cost of space includes land, building, tank, structure and tray costs. The relationships between equipment capacity (e.g., flow rate, heating rate) and capital cost include the effect of scale (Allen and Johnston, in press). The costs of waste treatment also include the effect of scale and are based on flow rate treated (9). The costs of the labor include cleaning, stocking, transfers, feeding, and harvesting. Food cost is based on costs of artificial rations available for other species ($0.65 /kg).

Cost estimates of system components are being refined and compared to current vendor prices.

The status of research and the impact on culture costs due to proposed advances through research are currently being evaluated. The advantages of increased growth rate from artificial genetic selection and eyestalk ablation are among these advances. The concept of communal rather than individual rearing for a portion of the culture period is also being evaluated.

The favored type of culture is currently the stacked tray, but this may change as other types are developed and compared. The projected culture time from fifth stage to 454 grams is 29 months for brine shrimp fed animals, at 21^{o}C in unlimited space. Assuming artificial rations can produce a growth rate 0.9 times that on brine shrimp, culture time is 32 months. For a system in which production cost is minimized, container size limits growth by approximately 30 per cent, increasing culture time to 42 months (Fig. 9). The optimal values of temperature and recirculation rate depend critically on relative costs of heating water and large scale waste treatment. If heating costs are reduced significantly (using an alternative to fossil fuel) a flow-through system at 21^{o}C will probably be optimal. This conclusion depends heavily on the degree of waste treatment required at the outflow by EPA and other agencies.

The current total cost estimate of lobster production in North America in a stacked tray configuration with a mechanical feeder is near six dollars for a 454 g lobster, of which three dollars is devoted to fossil fuel heating of water (Fig. 10). The latter cost can be reduced to less than 0.5 dollars using thermal effluent from a power plant. These

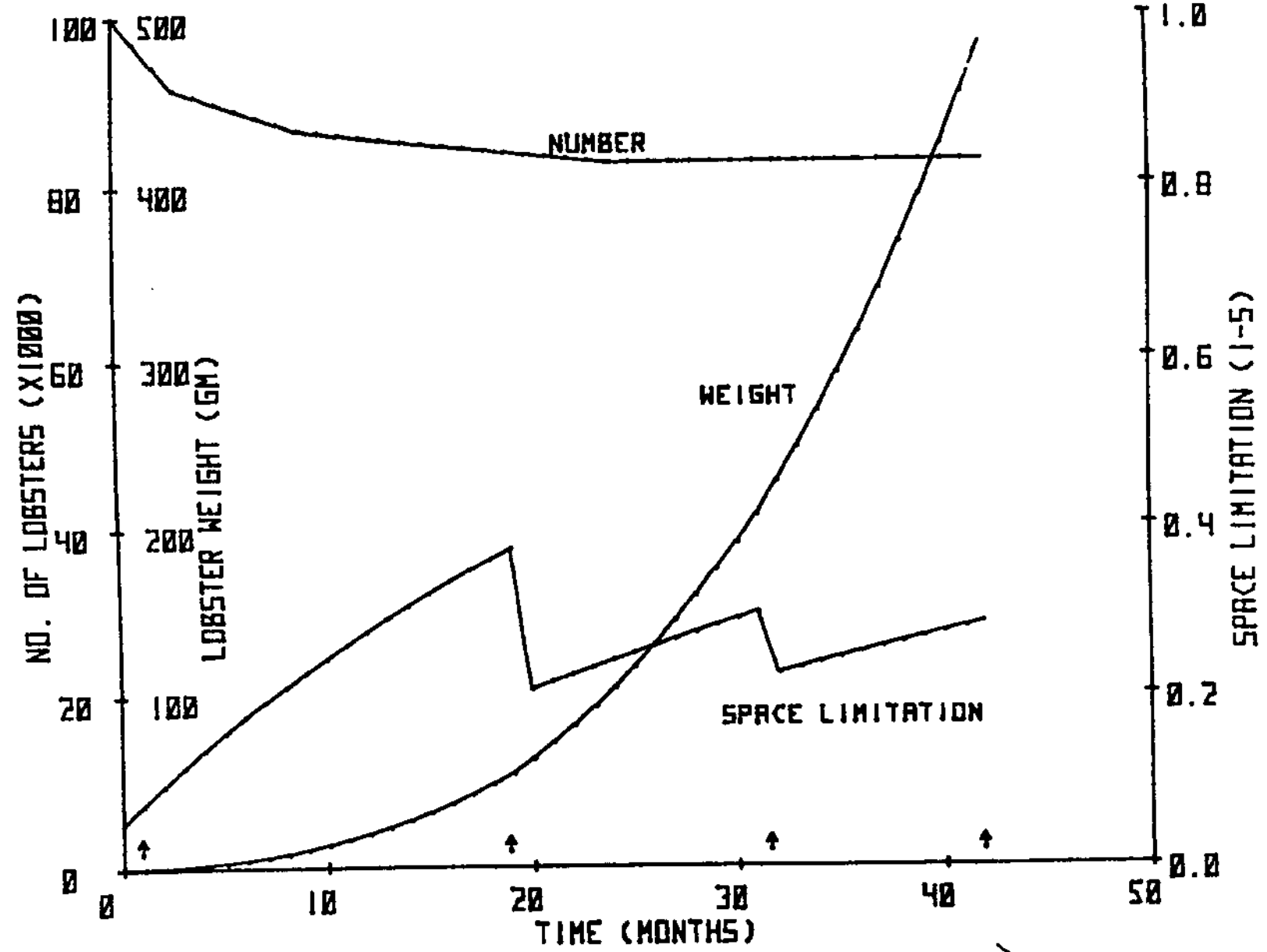

Figure 9. Number in one batch, weight of an individual and the degree of limitation of growth rate due to container size. Limitation increases as animal grows in one container then decreases when transferred to a larger container (↑). Container sizes are .013, .037, and .060 m^2.

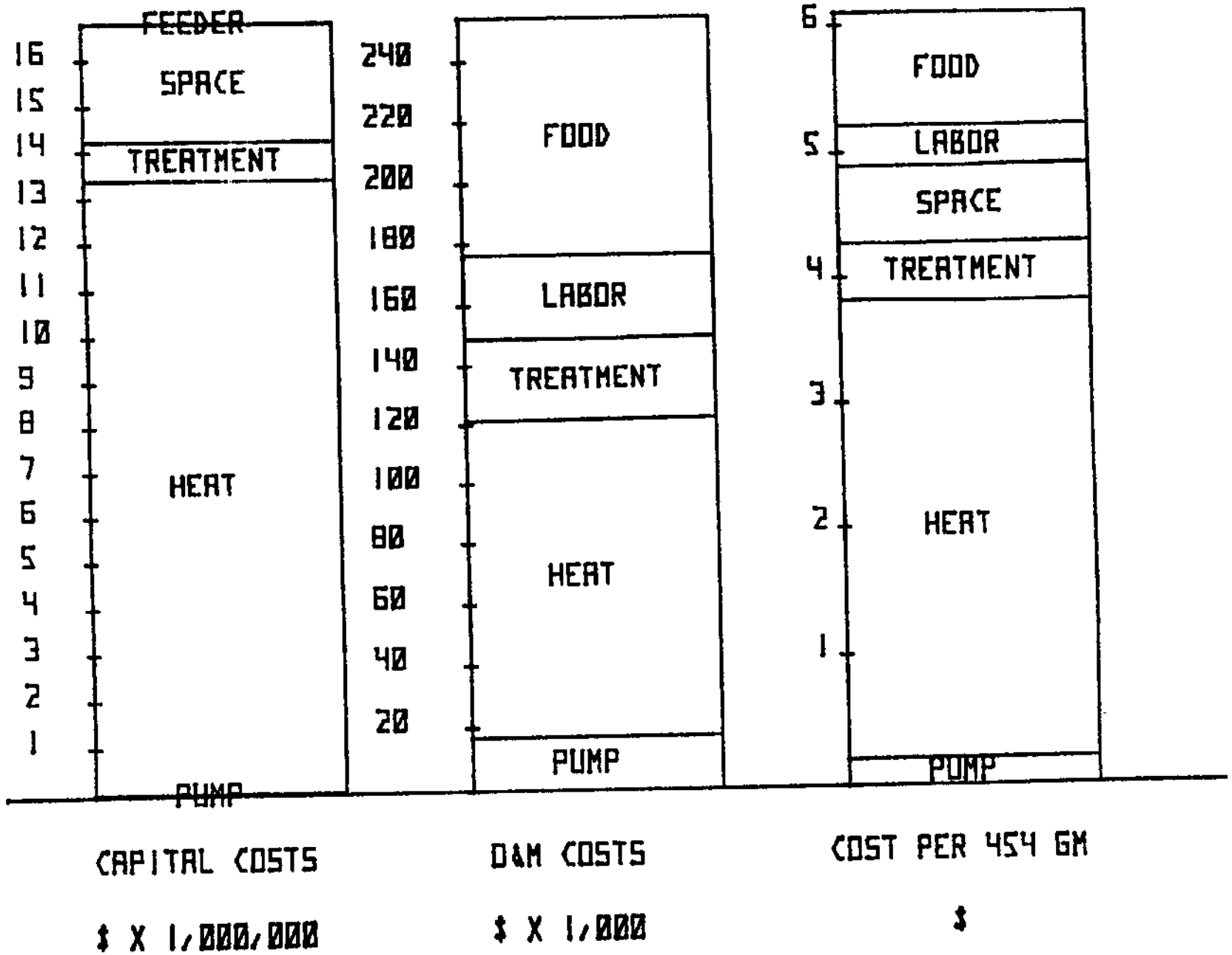

Figure 10. Distribution of capital costs, operating and maintenance cost and cost per 454 g lobster.

cost estimates depend heavily on the costs of components, the system configuration, and other external parameters. The model is basically a research tool rather than the final design of a culture facility.

A prospectus for a specific commercial facility must be based on the costs of equipment, labor and energy, legal requirements and ambient conditions of the geographical location of interest. The modeling approach and, more specifically, the models and techniques of analysis developed in the system analysis and economics program of this project can be very useful in this regard.

In summary, the major contribution of this program area is a paradigm for economic analysis of a lobster culture system. This includes a general model of most conceivable lobster culture situations, techniques for design of an optimal facility, and a means of projecting costs and directing research together with examples of their application.

CONCLUSION

No insurmountable physical or biological problems now stand in the way of lobster aquaculture. Lobsters can be grown with minimal mortalities from eggs to adults and our research has made substantial contributions to essentially all phases of the culture of these desirable food organisms. We have successfully resolved the technological problems associated with the design, construction and management of seawater systems for hatching, larval rearing and grow-out, and we have developed techniques for modeling the aquaculture of the lobster which account for both the biological properties and physical systems involved. The models are structured in an economic context. The cost projections from our studies indicate, however, that we are not yet at a time when the price of commercially raised lobsters can compete favorably with those caught by the commercial fishery.

We have made real progress toward the domestication of the lobster and an understanding of its biology. We have developed excellent data on growth in culture systems, nutrition, and genetics. We have defined the water-quality requirements of lobsters and can control all diseases which have appeared. Our work with artificial diets has shown continual progress, and we have had successes with mating, egg extrusion and subsequent hatching of lobsters in our laboratories. The two major problems still unresolved are those of a superior artificial diet and full control of the reproductive activities.

We believe that much of the data and technology we have developed for lobsters is transferrable to the aquaculture of other species. Our

experience with systems design and management for lobsters allowed us to develop systems for prawn and crab research quite readily. Configurations of the particular holding units may change from species to species, but the principles of ultraviolet treatment, pumping, filtration, temperature control, and recirculation remain the same. The work in nutrition has provided diets which may be used as standards for crustacean research and the diseases identified and controlled have broad applications to other crustacean species. Our water-quality and water-chemistry work is broadly applicable to marine aquaculture, and our genetic studies have developed the concept of biochemical tagging which will have wide applications in crustacean hatchery and culture programs, and has gathered data from the study of variation in many crustacean species in order to establish rules for candidate selection in aquaculture. Although our mathematical model was developed specifically for lobster culture, it can be modified to apply to culture of other species. The same techniques of cost projection, system optimization, and research direction can be usefully applied to any culture system. Overall, our findings appear generalizable to crustacean aquaculture as a whole.

Our approach has been advantageously multidisciplinary and has expedited our work. It has allowed for a division of labor and a sharing of expertise. The experience and knowledge gained in one area has decreased the likelihood of the repetition of errors by workers in other areas because of the close daily contact among our researchers and the joint nature of many of our actual experiments. Too, cross-comparisons between the several research areas have pointed up problems and generated ideas, and, because of the breadth of area and knowledge represented

by our multidisciplinary group, immediate discussion, and even experimental testing of hypotheses or problems,has been possible. In real ways and in terms of our accomplishments, what we have produced collectively is significantly greater than the output could have been if the individual research activities had been located at disparate institutions. Truly, the total is greater than the sum of the parts.

SEA GRANT TRAINEES

A number of graduate students have received support as Sea Grant Trainees over the five years covered by this report. Their names and the years concerned are listed below (all UC Davis except as noted).

1971-72

none

1972-73

Steve Nelson
Warren Flint
Steve Serfling
Walter Sadler
Lou Botsford

1973-74

Dan Wickham (UC Berkeley)

1974-75

Steve Loomis
William Krage
Dan Wickham (UC Berkeley)

1975-76

Dan Wickham (UC Berkeley)

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Tang, A.H., 1974. Utilizing various protein levels in a formulated feed on juvenile prawns, Pandalus platyceros (Brandt). Master's thesis, Chinese University of Hong Kong.

COOPERATING ORGANIZATIONS AND INDIVIDUALS

California Department of Fish and Game, Granite Canyon Laboratory, Monterey Laboratory, and Menlo Park Laboratory, California - Earl Ebert, Randy Kelley, Art Hazeltine, Hal Orcutt

National Marine Fisheries Service, Seattle, Washington - Dr. Fred Utter; Auke Bay, Alaska - John Karnin

Fisheries Research Board of Canada, Nanaimo, B.C. - Dr. Terry Butler

Humboldt State University, Arcata, California - Dr. William Allen, Dr. George Allen

Dr. Duane Brown and Dr. Allen Knight, aquaculture researchers at UC Davis

Dr. Richard Ford, Jim Carlberg, and Jack Van Olst, aquaculture researchers at San Diego State University

San Francisco Bay Brand Brine Shrimp Co., San Francisco, California

Phil Wilson, Monterey, California, commercial lobster culture

Dr. C.E. Bland, East Carolina University, North Carolina

Drs. H. Shapiro and J.F. Steenbergen, San Diego State University, California

Dr. S.W. Wellings, School of Medicine, UC Davis

State of California, Regional Water Quality Control Board

The Department of Land, Air and Water Resources, UC Davis

The Department of Soils and Plant Nutrition, UC Davis

Dr. J.S. Cobb, University of Rhode Island

Foremost Research Center, Dublin, California - Dr. Barry Holtz

Agromarina de Panama, S.A., Panama - Tony Schuur

A. Tang, Chinese University of Hong Kong

Walter Blogoslawski, Ozone Institute, Boston, Massachusetts

Bolsa Aqua, Huntington Beach, California - Max Schrieber, Bernard Cohlin

H & M Wholesale Lobster, Petaluma, California - Ernie Holmes

Fishermen's Marketing Association, Bodega Bay, California

Cooperating Organizations and Individuals (continued)

International Shellfish Enterprises, Moss Landing, California - Andy Zorbas

Ralston Purina, Crystal River, Florida and St. Louis, Missouri - William McGrath

Sanders Associates, New Hampshire - Paul Chapman and Emile Plante

University of California, Santa Barbara - Drs. Adrian Wenner and Ann Busath

University of Hawaii - Spencer Malecha and Gail Kotaji

University of Delaware - Dr. R. Srna, Mr. G. Pruder, and Dr. K. Price

Drs. Warren Johnston and P.G. Allen, Agricultural Economics, UC Davis

John Hughes, Massachusetts State Lobster Hatchery, Martha's Vineyard, Mass.

Dr. John West, Dept. of Botany, University of California, Berkeley

Marine Nutritional Systems, Denver, Colorado - J.E. Kitchel

Syntex, Palo Alto, California - Myron Beigler

Department of Food Science, Louisiana State Univ., Baton Rouge, Louisiana

Dr. Don Lightner, University of Arizona

Dr. F.J. Ayala, Department of Genetics, UC Davis

Dr. J.W. Valentine, Department of Geology, UC Davis

Ray Dunaway, commercial holding, Los Angeles, California

Dr. Carl Sindermann, National Marine Fisheries Service, Middle Atlantic Coastal Fisheries Service

Dr. H.E. Rauch, Lockheed Research Laboratory, Palo Alto, California

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